From: Staples, Rose
BCC To: Don Pedro Relicensing Participants Email Group
Sent: Friday, January 30, 2015 1:20 PM
Subject: Don Pedro Study Report for your Review and Comment

Please find attached for your review and comment a study report entitled *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature.*

This study was conducted as part of W&AR-14: Assessment of Temperature Criteria for the Don Pedro Hydroelectric Project by a team of UC Davis scientists under the direction of Dr. Nann Fangue and Dr. Anthony Farrell (University of British Columbia). The researchers investigated the thermal performance of juvenile Tuolumne River *O. mykiss* with respect to the seasonal maxima water temperatures the fish experience during the summer months. The UC Davis Team and Dr. Farrell tested wild locally caught *O. mykiss* in a swim tunnel respirometer as described in the report.

Please provide any comments you may have by March 2, 2015 to me at rose.staples@hdrinc.com.

Thank you.

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THERMAL PERFORMANCE OF WILD JUVENILE *ONCORHYNCHUS MYKISS* IN THE LOWER TUOLUMNE RIVER: A CASE FOR LOCAL ADJUSTMENT TO HIGH RIVER TEMPERATURE

REPORT
DON PEDRO PROJECT

Prepared for:
Turlock Irrigation District – Turlock, California
Modesto Irrigation District – Modesto, California

January 2015

Thermal Performance of Wild Juvenile *Oncorhynchus mykiss* in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature

Prepared for:

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January 2015

EXECUTIVE SUMMARY

The purpose of this study was to investigate the thermal performance of juvenile *Oncorhynchus mykiss* that populate the lower Tuolumne River in the Central Valley region of California with respect to the seasonal maxima water temperatures they experience during the summer months.

The study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer. The basis for this hypothesis is peer-reviewed scientific literature that indicates that salmonid species, including *O. mykiss*, can adjust to local thermal conditions. In the current study, *O. mykiss* were locally caught and tested, and then returned safely within ~ 1 day of capture to the Tuolumne River.

The experimental approach acknowledged the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, which proposes that the extremes of thermal tolerance are set by a fish's inability to supply oxygen to its tissues above and beyond a basic routine need. The tests performed here directly measured how much oxygen can be maximally extracted from the water by a fish (its maximum metabolic rate; MMR) and how much oxygen is routinely needed by that fish to exist (its routine metabolic rate; RMR). These measurements were performed in a swim tunnel respirometer (the equivalent of an aquatic treadmill) at different test temperatures ranging from 13°C to 25°C. By subtracting RMR from MMR, we determined over this temperature range the capacity of *O. mykiss* to supply oxygen to tissues above and beyond a basic routine need, which is termed the absolute aerobic scope (AAS = MMR - RMR) and defines the fish's capacity to perform the activities essential to complete its life history. Factorial aerobic scope (FAS = MMR/RMR) was also calculated and is another way of expressing a fish's aerobic capacity. Therefore, the experimental approach also acknowledged that every activity of a fish in a river (swimming, catching prey and feeding, digesting a meal, avoiding predators, defending territory, etc.) requires oxygen above and beyond a basic routine need and that salmonids have evolved to maximize their oxygen supply when they fuel muscles during exhaustive swimming,

As expected for a fish, RMR increased exponentially with test temperature from 13°C to 25°C (36 different fish, each at a single test temperature). For these same fish, MMR also increased over the same range of test temperatures, but to a lesser degree. As a result, the average AAS (as modeled for all fish by a mathematical equation) reached a peak at 21.2°C. The statistical 95% confidence limit for peak AAS extends between 16.4°C and 25°C. Likewise, 95% of the numerical peak for AAS (i.e., 5.84 mg O_2 $kg^{-0.95}$ min^{-1}) could be maintained from 17.8°C to 24.6°C. Thus, the maintenance of AAS across nearly the entire test temperature range clearly shows that the Tuolumne River *O. mykiss* population has a broad range of thermal performance. Indeed, the AAS of the Tuolumne River *O. mykiss* population was atypical when compared with cold-adjusted, *O. mykiss* from the Pacific northwest, whose thermal performance optimum is reported as 18°C (EPA, 2003). The upper thermal performance limit (i.e., the temperature where AAS is zero) for Tuolumne River *O. mykiss* was not determined due to conditions set forth by the National Marine Fisheries Service (NMFS), but must lie above 25°C based on the present data.

This report supports the hypothesis that the *O. mykiss* population found in the Tuolumne River downstream of La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that typify the summer months. Indeed, all fish recovered quickly from the exhaustive swim test and were successfully returned to the river, with the exception of one of the four fish tested at 25°C. Some of the test fish were inadvertently recaptured up to 11 days later in their original river habitat and appeared to be in excellent condition when visually inspected.

The conclusion of the study is that the thermal range over which the Tuolumne River *O. mykiss* population can maintain 95% of peak aerobic capacity is 17.8°C to 24.6°C. Moreover, up to a temperature of 23°C, all individual fish could maintain a FAS value >2.0, one that is predicted to provide sufficient aerobic capacity for the fish to properly digest a meal. Finally, based on a video analysis of the swimming activity of *O. mykiss* in the Tuolumne River, fish at ambient water temperatures were predicted to have an excess aerobic capacity well beyond that needed to swim and maintain station against the river current in their usual habitat.

These results support the hypothesis that the thermal performance of wild *O. mykiss* from the Tuolumne River represents an exception to that expected based on the 7DADM criterion set out by EPA (2003) for Pacific northwest *O. mykiss*. Moreover, given that the average AAS remained within 5% of peak performance up to a temperature of 24.6°C and that all Tuolumne River *O. mykiss* maintained a FAS value >2.0 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, be used to determine a 7-Day Average of the Daily Maximum (7DADM) value. This thermal performance is consistent with that found for *O. mykiss* populations already known to be high-temperature tolerant, such as the redband strain of rainbow trout (*O. mykiss gairdneri*) in the high deserts of Eastern Oregon and Idaho, as well as selected and hatchery-maintained strains of *O. mykiss* in Western Australia and Japan, as well as steelhead trout from the south coast of California. Whether the high thermal performance that was demonstrated for the *O. mykiss* of the Tuolumne River downstream of La Grange Diversion Dam arose through genetic selection or physiological acclimatization was beyond the purpose and scope of the present study.

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7DADM	7-Day Average of the Daily Maximum
95% CI	95% Confidence Limits
AAS	Absolute Aerobic Scope (MMR-RMR)
AS	Aerobic Scope
BP	Barometric pressure
CTmax	Critical Thermal maximum
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act
FAS	Factorial Aerobic Scope (MMR/RMR)
FL	Fork Length of the fish
ILT	Incipient Lethal Temperature
M	Mass of fish
MMR	Maximum Metabolic Rate
MR	Metabolic Rate
O_2	Oxygen
O_2(A)	Tunnel water oxygen concentration at beginning of seal
O_2(B)	Tunnel water oxygen concentration at end of seal
OCLTT	Oxygen- and Capacity-Limited Thermal Tolerance
PIT	Passive Integrated Transponder
RM	River Mile
RMR	Routine Metabolic Rate
T	Time
TBF	Tail Beat Frequency
T_{crit}	Critical Temperature when performance (e.g., aerobic scope) reaches zero
T_{opt}	Optimal Temperature when performance (e.g., aerobic scope) reaches a peak
T_p	Pejus Temperature when performance (e.g., aerobic scope) decreases from its peak. In the present study, T_p is set when absolute aerobic scope decreases to 95% of the peak capacity at T_{opt}
V	Tunnel volume
$\alpha(O_2)$	Solubility of oxygen in water
% O_2Sat	Percent saturation of oxygen in water

INTRODUCTION

The Tuolumne River has been significantly affected by human activity since the mid-1800s, including in-channel and overbank mining of gold and gravel, urban and agricultural encroachment, and water resource development. Summertime water diversions from the Tuolumne River near La Grange, CA have been occurring for over 120 years. These changes have contributed to a unique river habitat for the *O. mykiss* population that lives in the Tuolumne River downstream of the La Grange Diversion Dam located at River Mile 52 (RM 52). Year round, the Don Pedro Dam located near RM 54 releases cool water to the river (10-13°C) even during the hottest periods in summer. As this water flows downstream it can gain or lose thermal energy depending on its surrounding environment. In summer months, the average river temperature increases appreciably with distance downstream of the dam (see Appendix 1). At RM 49, for example, river temperature peaked at 20.2°C in July 2014. However, cooler river temperatures are associated with cloud cover and over night, and deeper ponds in the river do show some thermal stratification. In 2013, a detailed study of summertime temperatures in the Tuolumne River was performed between ca. RMs 3-37 (HDR 2014).

Based on observations from monitoring surveys conducted since 1997 (Ford and Kirihara 2010; Stillwater Sciences 2012), *O. mykiss* rearing habitat extends from RM 52 to ca. RM 30, with spawning habitat in 2013 documented from RM 50 to about RM 39 (FISHBIO 2013). Review of this information suggests that primary rearing habitat for *O. mykiss* since 1997 has been concentrated upstream of RM 39.6, where peak water temperatures have occasionally exceeded 27°C during the summer months. Therefore, the realized habitat of *O. mykiss* during summer presently covers a distance of ca. 12.4 river miles, where water temperature varies within the range of 11°C to 28°C. Any difference between where a fish actually lives (the realized habitat) and its fundamental habitat is determined by behavior (Matthews and Berg 1997).

Thermal Tolerance and Thermal Performance

Fundamental habitat of any animal is determined by its thermal tolerance limits to warm and cold. Even humans, who normally regulate body temperature at 37°C (98.4°F), succumb if body temperature cannot be maintained below 45°C in extreme heat. However, the body temperature of a fish such as *O. mykiss* in the Tuolumne River is not regulated in the same way as that of humans. Instead, it is always the same as the surrounding river temperature, except for brief (seconds to minutes), non-equilibrium states whenever a fish moves rapidly between regions of thermal stratification. Nevertheless, a fish warmed or cooled beyond its thermal limits will rapidly succumb, just like a human.

Scientists commonly measure the thermal tolerance limit of a fish using either incipient lethal temperature (ILT) or critical thermal maximum (CTmax) tests. An upper ILT test acutely exposes fish to a suite of elevated temperatures and reports the temperature at which 50% of the test fish succumb. In contrast, an upper CTmax test warms (ca. 0.3°C per min) a fish until it can no longer maintain its upright orientation and reports the temperature when 50% of the fish roll over.

While CTmax values have been widely used to distinguish thermal tolerance differences among fish species, CTmax does not always discriminate more subtle physiological adjustments in thermal tolerance expected within a fish species in response to season and/or genetic differences. For example, a CTmax value of 29°C is reported for trout acclimated to temperatures ranging from 12 to 20°C (Table 1). While CTmax values for *O. mykiss* can certainly be similar over a wide range of thermal acclimation temperatures and populations, there are exceptions because CTmax has been shown to increase with thermal acclimation in *O. mykiss* (Table 1), as it does in killifish (Fangue et al. 2006), and the sub-species redband trout has the highest CTmax for the genus. Any insensitivity of the CTmax measurement likely stems from relatively short test exposure times (min) and the rapid but sometimes variable warming rates that are employed. Regardless, CTmax is always higher than the temperature that a fish can tolerate for hours to days and certainly higher than the temperature at which a fish can no longer swim aerobically.

Consequently, despite the relative ease of measurement, fish biologists are increasingly replacing CTmax, which is a measure of thermal tolerance, with metrics that measure thermal performance, especially if the metric has some ecological relevance, such as growth and the fish's ability to deliver oxygen (O_2) to its tissues. While methods to characterize fish thermal performance date back some 60 years (e.g., Fry 1947), watershed managers have only embraced thermal performance metrics for about a decade. As a result, historically there has been a natural and reasonable tendency to simplify regulatory criteria in addition to setting conservative limits.

7-day Average of the Daily Maxima (7DADM)

One of the thermal criteria used by EPA to protect fish is the 7-day average of the daily water temperature maximum (7DADM). The explicit recommendation in EPA (2003) for juvenile *O. mykiss* in summer rearing habitats is a 7DADM <18°C. A key study that influenced the current 7DADM criterion for *O. mykiss* from the Pacific northwest is the growth study of Hokanson et al. (1977), which was reviewed in Issue Paper 5 (EPA 2001). Growth is considered as a very powerful integrator of environmental, behavioral and physiological influences of a fish's fitness.

Hokanson et al. (1977) measured growth of juvenile *O. mykiss* from the Great Lakes in Minnesota using constant and fluctuating (a daily temperature oscillation of ± 3.8°C) thermal regimes. *O. mykiss* grew maximally at 16-18°C, termed the optimum temperature (T_{opt}) for growth. However, Hokanson et al. (1977) did not place a statistical 95% confidence interval (CI) around the temperature for peak growth. This is an important data gap because EPA (2003) states that: "*Each salmonid life stage has an optimal **temperature range** (our emphasis). Physiological optimum temperatures are those where physiological functions (e.g., growth, swimming, heart performance) are optimized. These temperatures are generally determined in laboratory experiments.*" Therefore, this key study did not establish a thermal range for peak growth performance. Interestingly, by setting the 7DADM criterion for salmon and trout migration as 20°C, rather than 18°C, EPA (2003) acknowledged that juvenile Pacific Northwest *O. mykiss* have sufficient aerobic scope for the energetic demands of river migration even at a temperature 2°C above the 7DADM for juvenile growth.

Hokanson et al. (1977) also discovered that "*At temperatures in excess of the growth optimum, mortality rates were significantly higher during the first 20 days of this experiment than the last 30 days.*". The implication of this observation is that a proportion of the test fish were either initially better suited for high temperature or became better suited after living for 20 days at a supra-optimal temperature when compared to the fish that died during the initial 20-day period. While this core study used by the EPA acknowledged the possibility for local physiological acclimation or genetic adaptation to warm temperature within the *O. mykiss* genus, extensive evidence now exists for similar adjustments to local conditions.

Current Evidence for Local Physiological Acclimatization and Genetic Selection

As early as the late 1960s, Bidgood and Berst (1969) used upper ILT data to conclusively demonstrate that juvenile *O. mykiss* from four anadromous Great Lakes populations could thermally acclimate, i.e., warm acclimation increased their upper ILT. Indeed, the extensive knowledge on thermal acclimation among fish species dates back well into the 1940s. In California (CA) there is wide variation in the thermal performance curves for hatching success among different strains of *O. mykiss* (Myrick and Cech 2001). While this variability includes the Eagle Lake and Mt. Shasta strains, these two strains had been shown earlier to have a similar CTmax (Myrick and Cech 2000). Thus, in the early 2000s, evidence for thermal acclimation was extensive for *O. mykiss,* but evidence for thermal adaptation was limited.

Issue Paper 5 (EPA 2001) did ask the question of whether there is enough evidence for genetic variation within a species to warrant geographically-specific or stock-specific water temperature standards. The conclusion was “The literature on genetic variation in thermal effects indicates occasionally significant but very small differences among stocks and increasing differences among subspecies, species, and families of fishes. Many differences that had been attributed in the literature to stock differences are now considered to be statistical problems in analysis, fish behavioral responses under test conditions, or allowing insufficient time for fish to shift from field conditions to test conditions”.

Actually, Issue Paper 5 (EPA 2001) cites (see its Table 1) Sonski (1983), who identified the T_{opt} for growth of redband trout (*O. mykiss gairdineri*) as 20°C, which is the highest value for the genus *O. mykiss*. Therefore, evidence did exist that the genus *O. mykiss* can perhaps be genetically adapted to local environmental conditions. However, since 2001, thermal adaptation at the population level and among a wide variety of fish species (e.g., killifish populations on the Atlantic coast, Fangue et al., 2006; stickleback populations in the Pacific northwest, Barrett et al., 2011) has been convincingly supported in the peer-reviewed scientific literature. Included in this evidence base are salmon and trout species. For example, Eliason et al. (2011) showed that populations of adult sockeye salmon in British Columbia’s Fraser River watershed are adjusted to perform best at the local temperature conditions that they experience during their spawning river migration. Indeed, their swimming capacity is also well matched with the hydraulic challenge that they face migrating upstream to their spawning area (Eliason et al. 2013). New thermal performance studies in redband trout from a desert population in eastern Oregon, where stream water temperatures can exceed 30°C, provide additional evidence for local thermal adaptation (Rodnick et al. 2004). Moreover, redband trout’s ability to genetically adapt when acclimated to a common set of experimental conditions has found support (Narum et al. 2010, 2013). In addition, selective breeding of the *O. mykiss* genus has been effective in selecting for high temperature tolerance. For example, severe thermal exposures in a hatchery program in Western Australia have produced a line of *O. mykiss* that is thermally tolerant (Morrissy 1973;

Molony 2001; Molony et al. 2004; Chen et al. submitted). During summer extremes, the juvenile *O. mykiss* swim and feed at a water temperature of 26°C (Michael Snow, Department of Fisheries, Government of Western Australia, pers. comm.). The founder *O. mykiss* population for this thermally tolerant line had been transplanted from CA for recreational fisheries during the last century. Japanese researchers have similarly selected a strain of rainbow trout that show high thermal tolerance (Ineno et al., 2005).

Therefore, clear and compelling scientific knowledge exists for local adjustments and genetic selection of high thermal performance of *O. mykiss*. This new knowledge has been largely added to the scientific literature after the 18°C 7DADM for *O. mykiss* in the Pacific northwest was established as a result of the EPA (2003) report. In fact, the EPA (2003) report did acknowledge that local adjustment was possible and that well-designed studies could be used to identify site-specific thermal adjustments.

Justification and Purpose of the Study

The primary purpose of this study is to determine the thermal performance of the subadult (100-200 mm fork length; FL) *O. mykiss* population inhabiting the lower Tuolumne River (LTR) to assess any local adjustment in thermal performance. Thermal performance was assessed as the range of temperatures over which juvenile *O. mykiss* can increase aerobic metabolic rate (MR) beyond basic needs. This aerobic capacity could be used for any of the normal daily activities of *O. mykiss* in the Tuolumne River during its normal life history (swimming, catching prey and feeding, digesting a meal, growing, avoiding predators, defending territory, etc.). Thus, MR measurements were used to determine the optimal temperature range for Tuolumne River *O. mykiss.*

This experimental approach is consistent with the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis that has emerged as a conceptual model to assess thermal performance of aquatic animals and determine the fundamental thermal range for their distributions (Pörtner and Knust 2007; Pörtner and Farrell 2008). The OCLTT hypothesis proposes that the extremes of thermal tolerance will be set by a fish's inability to supply oxygen to its tissues above a basic routine need.

Salmonids are examples of fish that have evolved to maximize oxygen supply to exhaustive swimming muscles. Therefore, our experimental approach directly measured MR under two states: routine metabolic rate (RMR), representing how much oxygen is needed by an individual *O. mykiss* to exist in the Tuolumne River and maximum metabolic rate (MMR), representing how much oxygen can be maximally extracted from the water for its tissues, typically when swimming. The capacity of the fish to supply oxygen to tissues above and beyond a basic routine need is then calculated by subtracting RMR from MMR, which is termed the absolute aerobic scope (AAS = MMR - RMR). Therefore, AAS defines a fish's capacity to perform the activities essential to carry out its life functions. Factorial aerobic scope (FAS = MMR/RMR) is another way of expressing aerobic capacity by characterizing how much a fish can increase its RMR. Necessary activities for survival in nature like feeding and digestion are expected to require up to a doubling of a fish's RMR (Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008), which would require a FAS value of 2.

Measurements of fish MR were obtained using the equivalent of an aquatic treadmill (a swimming tunnel respirometer) and at different test temperatures (from 13°C to 25°C). By mathematically modeling these data, the optimal temperature (T_{opt}) for the peak AAS could be established for juvenile *O. mykiss*. The T_{opt} window (or thermal range) is defined by Parsons (2011) as *"the range in temperatures where maximum aerobic scope is maintained*". In the present study, we use 95% of the peak AAS value to set the optimal thermal range (Figure 1; the two temperatures that bracket T_{opt} are termed a Pejus temperature, T_p). If, as predicted by the OCTTL hypothesis, a cardiorespiratory limitation exists for exercising salmonids during warming, AAS will decrease below 95% of peak AAS beyond the upper T_p, and often rapidly over just a few degrees before lethal temperatures are reached (Farrell 2009). The critical temperature (T_{crit}) is the temperature when there is no aerobic scope and therefore aerobic activities beyond basic needs, including swimming, are impossible. Thus, whenever a fish is warmed beyond its T_p, maximum oxygen delivery progressively fails to quantitatively keep up with the need for increased oxygen delivery just to maintain the resting state (Farrell 2009). As a result, the factorial aerobic scope (AMR/RMR) will decrease with temperature. Should FAS decrease below a value of 2, the doubling of RMR needed for digestion (Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008) would not be possible.

Thus, the primary study goal is to determine if there is evidence for local temperature 'adjustment' in Tuolumne River *O. mykiss* by establishing the temperatures that set the thermal range for T_{opt} (at 95% of peak) and determining how rapidly AAS declines between the upper T_p and T_{crit} for Tuolumne River *O. mykiss*. This information should help define more accurate criteria for thermal performance of juvenile *O. mykiss* rearing in the lower Tuolumne River. Specifically, the temperature indices and the shape of the aerobic scope curve derived in the present study can also be compared with those of other *O. mykiss* populations and with the EPA (2003) recommendations.

While the curve relating AAS with temperature has been coined a Fry aerobic scope curve (Fry 1947), curves that describe the effect of temperature on a measure of organismal performance (e.g., RMR, MMR, AAS, growth) are more generally called thermal reaction norms (Huey and Kingsolver 1979; Schulte et al. 2011). Reaction norms typically have a shape in which the performance index increases with increasing temperature, reaches a peak at some intermediate temperature, and declines with a further temperature increase. Importantly, the specific shape and position of these performance curves can vary among species and in response to thermal variation in a fish's environment. The magnitude and timescale of environmental temperature exposure are both critical and persistent differences in local thermal conditions over evolutionary time scales may result in compensatory adaptive changes in local populations (Hochachka and Somero 2002). On a shorter time scale, and if temperature varies on a daily or seasonal basis at a given locality, fish may compensate for the temperature difference over weeks to months - termed thermal acclimatization for natural settings or simply thermal acclimation when only temperature is manipulated under controlled laboratory conditions. Fish can also respond immediately (seconds to hours) to acute thermal challenges using either behavioral (e.g., attraction and avoidance), or physiological and biochemical responses (e.g., changes in heart rate and heat shock proteins).

Although, the theoretical basis for how patterns of thermal performance can be shaped by local thermal regimes is now well understood and this theory provides the framework for the present study, our study was not designed to distinguish between the mechanisms of local thermal adaptation (which implies a proven genetic change) and acclimatization. Consequently, rather than using the term 'adaptive', we say that the fish are acclimatized to the local conditions and will use the general term that fish are 'well adjusted' to local environmental conditions, if we find that to be the case.

EPA (2003) also states that: "*Ecological optimum temperatures are those where fish do best in the natural environment considering food availability, competition, predation, and fluctuating temperatures. Both (sic lab-based and field based measurements) are important considerations when establishing numeric criteria.*" Importantly, Issue Paper 5 (EPA 2001) comments that "*Field testing of fish survival under high temperatures is not usually done. If such methods were feasible, the improved realism would be helpful.*" Therefore, the present experiments established a field laboratory beside the Tuolumne River so that the thermal performance of wild *O. mykiss* acclimatized to field conditions could be tested without prolonged transport and holding of fish.

Predictions and Alternate Predictions

Given the EPA (2003) 7DADM and the current scientific literature, it is possible to make two types of contrasting predictions for the upper thermal performance of wild *O. mykiss* captured from the Tuolumne River: a) predictions based on the EPA (2003) 7DADM criterion, and b) alternative predictions based on contemporary literature for local thermal adjustment.

Predictions Derived From EPA (2003)

Based on the EPA (2003) 7DADM criteria alone, one would predict that wild *O. mykiss* captured from the Tuolumne River for the present tests would show the following:

1. Routine metabolic rate (RMR) will increase exponentially until the test temperature approaches the upper thermal limit for *O. mykiss* (i.e., CTmax), which depending on the *O. mykiss* strain and acclimation temperature, is 26°C to 32°C (see Table 1).
2. Maximum metabolic rate (MMR) will increase with test temperature and reach a peak around 18°C according to the EPA criterion.
3. Absolute aerobic scope (AAS) has a T_{opt} around 18°C according to the EPA criteria.
4. AAS will decline at a temperature just above 18°C.
5. Factorial aerobic scope (FAS) will decline with increasing temperature, reaching a value < 2 (i.e., MMR is less than twice RMR) at a temperature just above 18°C.

Alternative Predictions of Thermal Adjustment

Based on recent peer-reviewed studies, the present study tested the hypothesis that the Tuolumne River *O. mykiss* population below La Grange Diversion Dam is locally adjusted to the relatively warm thermal conditions that exist in the river during the summer. One would then predict that the results of the present study would show the following:

1. RMR will increase exponentially until the test temperature approaches the upper thermal limit for *O. mykiss* (i.e., CTmax), which is ca. 26°C to 32°C depending on the study.
2. MMR will increase with test temperature and reach a peak that is above 18°C.
3. AAS will have a T_{opt} that is above 18°C.
4. AAS will decline at a temperature well above 18°C.
5. FAS will decline with increasing temperature, but maintain a value > 2 well above 18°C.

METHODS

Permitting Restrictions that Influenced the Experimental Design

Wild Tuolumne River *O. mykiss* were collected under National Marine Fisheries Service Section 10 permit # 17913 and California Fish and Wildlife Scientific Collecting Permit Amendments. No distinction was made between resident (rainbow trout) and anadromous (steelhead) life history forms, and both are referred to as *O. mykiss* throughout this document. For permitting purposes, these fish are considered as “ESA-listed California Central Valley steelhead, *O. mykiss*”.

Fish collection (to a maximum of 50 fish) was allowed between RM 52.2 and RM 39.5, and between June 1 and September 30, 2014. Fish collections were not allowed at river water temperatures that exceeded 70°F (21.1°C). Incidental fish recaptures were authorized in addition to the initial take limit (n=50), with these reported as ‘additional take’ under the NMFS permit reporting conditions. Because indirect fish mortality was limited to 3 fish, no more than 2 fish were captured per day as a precautionary measure to limit indirect mortalities. Also, temperatures were not tested randomly and most of the highest temperatures were tested last to preclude premature termination of the work should there be high-temperature related mortality.

Preliminary experiments were performed with hatchery reared *O. mykiss* to ensure that all the equipment was fully functional and properly calibrated prior to testing wild fish. All experimental procedures were approved by the University of California Davis’ Institutional Animal Care and Use Committee (Protocol # 18196). All fish capture and handling activities were conducted by experienced FISHBIO personnel.

Fish Collection, Transport, and Holding

Fish capture was conducted via seine net (0.32 cm nylon mesh, 1.8 m high, 9 m long). Several precautions were used during capture activities in order to minimize handling of non-target fish. These included 1-2 snorkelers in the water identifying *O. mykiss* of the target size range (100-200 mm) prior to seine sweeps, as well as the use of a mesh size allowing fish smaller than the target fork length to avoid capture. Captured fish within the target range were transferred to a partially submerged transport tank via a large scoop net to minimize handling and avoid air exposure during transfer. Each captured fish was scanned for presence of a PIT tag to ensure that the fish had not been tested previously. Upon capture, a water temperature logger (Onset Computer Corporation) was placed in the transport tank with the fish recording temperature at 15 min intervals through the duration of the fish holding/testing period. These loggers remained in the water with the fish throughout all transport, experimental protocols and handling until fish were returned to the river.

In total, 48 *O. mykiss* were captured between July 11 and August 13, 2014 (Appendix 2). Each fish was given a unique identification (‘W’ for wild, followed by a number between 01 and 48). Two fish were captured and tested daily using four capture locations (Figure 2). The fish ID, capture location (River Mile, RM), and any recaptures are shown in Figure 3 and summarized in Appendix 3. Most of the test fish (36) were captured from a single site (RM 50.7), 8 fish were captured at RM 51.6, 2 at RM 50.4 and 2 at RM 49.1 (Figure 3). Instantaneous water temperature and dissolved oxygen (DO) levels were recorded at the time of capture, and varied between 12.7 and 17.1°C. Temperature loggers were placed at RM 40, 42, 44, 46, and 48-50 from early June to late September, 2014. From the logged temperature data, 7DADM at each RM location was calculated and plotted in Appendix 1. Additional information about release locations, water temperatures, time of day, and general comments are summarized in (Appendix 2).

Fish were placed individually into 13-l plastic transport tanks, modified with many 0.8 cm diameter holes drilled at least 2.0 cm from the bottom to ensure sufficient water movement through the transport container. The fish, inside its transport tank, was placed into an individual insulated Yeti cooler filled with 25 l fresh river water and driven to the experimental field site. Water temperature and DO were re-measured in the transport tanks on arrival and fish were transferred from the coolers to outdoor holding tanks (300 l) filled with flow-through Tuolumne River water between 12.5 and 13.6°C. This approach allowed for water-to-water transfer to minimize handling stress.

The holding tanks received river water passed through a coarse foam filter then a 18-l gas equilibration column for aeration. This water was split between the holding tanks and the sump tank supplying the swim tunnels. Oxygen content in all vessels remained above 80% air saturation at all times. Time from fish capture in the river to placement into holding tanks ranged from 60 to 120 min. Fish remained in holding tanks for 60 to 180 min before being transfer to a swim tunnel respirometer.

Swim Tunnel Respirometry

Individual fish were tested in one of two, 5-l automated swim tunnel respirometers (Loligo, Denmark). As with the holding tanks, swim tunnels were supplied with Tuolumne River water but via a fine pressurized 20-µm pleated filter; then a 180-l temperature-controlled sump, which operated as a partial recirculating system; and an 18-l gas equilibration column. The sump was continuously refreshed with air-equilibrated river water, turning over the entire system every 80-90 min. Additionally, an aquarium grade air pump supplied air stones in each tunnel bath for aeration. For temperature control, water from the sump was circulated through a 9500 BTU Heat Pump (Aqua Logic Delta Star. Model DSHP-7), and returned to the sump through a high volume pump (model SHE1.7, Sweetwater®, USA), where two proportional temperature controllers (model 72, YSI, Ohio) were mated to one 800 Watt titanium heater each (model TH-0800, Finnex, USA), resulting in temperature control precision of ± 0.5°C across a temperature range of 12 to 26 °C. To prevent buildup of ammonia waste in the water, ammonia-absorbing zeolite was kept in the system's sump and replaced weekly. Swim tunnel water baths were refreshed with the aerated sump water approximately every 20 min.

Water oxygen saturation was monitored using dipping probe mini oxygen sensors, one per tunnel, connected to AutoResp software through a 4-channel Witrox oxygen meter (Loligo). Water temperature in the swim tunnel was monitored with a temperature probe connected through the Witrox system and temperature loggers (see Fish collection, transport, and holding).

To limit disturbance of fish, swim tunnels were enclosed with black shade cloth. Above each tunnel, video cameras with infrared lighting (Q-See, QSC1352W, China) were mounted to continuously monitor and record (Panasonic HDMI DVD-R, DMR-EA18K, Japan) fish during swims and overnight routine metabolism measurements.

Measuring Metabolic Rates

All routine and swimming metabolic rates were measured using intermittent respirometry. A flush pump connected each tunnel chamber with an aerated external bath to allow control of tunnel sealing (during oxygen measurements) and flushing with fresh, aerated water. The pump was controlled automatically through AutoResp software and a DAQ-PAC-WF4 automated respirometry system (Loligo).

When the flush pump for the swim tunnel was off, no gas or water exchange occurred within the tunnel and so the oxygen level in the tunnel water declined due to fish respiration. Therefore, the rate at which oxygen declined in the tunnel was an estimate of aerobic metabolism. Oxygen drop (in mg O_2) was calculated for a minimum 2 min period when the tunnel was sealed. To restore oxygen levels in the swim tunnel, a flush pump connected to the external water bath refreshed tunnel water for periods of 2 to 5 min. Oxygen levels were never allowed to fall below 80% saturation.

Two-point temperature-paired calibrations at 100% and 0% oxygen saturation were performed weekly on the oxygen probes. The 100% calibration was performed in aerated distilled water. The 0% calibration was performed in 150 ml distilled water with 3 g of sodium sulfite (Na_2SO) dissolved. Swim tunnels were bleached and rinsed weekly. At the beginning and end of the 2-month experiment, background oxygen consumption measures of both tunnels without fish were performed. No oxygen consumption for these controls, even at the highest test temperature (25°C), was detected.

Percent saturation was converted to oxygen concentration ($[O_2]$, mg O_2 l^{-1}) using the formula:

$$[O_2] = \%\ O_2Sat/100 \times \alpha(O_2) \times BP.$$

Where $\%O_2Sat$ is the percent oxygen saturation of the water read by the oxygen probes; $\alpha(O_2)$ is the solubility coefficient of oxygen in water at the water temperature (mg O_2 l^{-1} $mmHg^{-1}$); BP is barometric pressure in mmHg.

Metabolic rate (MR in mg O_2 $kg^{-0.95}$ min^{-1}) for resting and swimming fish was calculated according to the formula:

$$MR = \{[(O_2(A) - O_2(B)) \times V] \times M^{-0.95}\} \times T^{-1}$$

Where $O_2(A)$ is the oxygen concentration in the tunnel at the beginning of the seal (mg O_2 l^{-1}); $O_2(B)$ is the oxygen concentration in the tunnel at the end of the seal (mg O_2 l^{-1}); V is the volume of the tunnel (l); M is the mass of the fish (kg); T is the duration of the seal (min).

To account for individual variation in body mass, MR was allometrically corrected for fish mass using the exponent 0.95. This value is halfway between the life-stage-independent exponent determined for resting (0.97) and active (0.93) zebrafish (Lucas et al. 2014).

Experimental Protocols

Fish were placed individually into the swim tunnels between 1300 h and 1600 h on the day of capture. Water temperature in the swim tunnels was set to 13 ± 0.3°C and fish were given a 60 min adjustment period to this temperature prior to a 60 min training swim. Each tunnel was equipped with a variable frequency drive motor designed to generate a laminar water flow through the swimming section of the tunnel (calibrated to water velocity using a digital anemometer with a 30-mm vane wheel flow probe; Hönzsch, Germany). During the training swim, water flow velocity was gradually increased until the fish moved off of the tunnel floor and began to swim (usually at ca. 30 cm s^{-1}). Once the fish began swimming, water velocity was further increased to 5-10 cm s^{-1} above the initial swimming speed and held for 50 min. To complete the training swim, for the last 10 min of the training swim water velocity was increased to a maximum of 50 cm s^{-1}, which is the expected maximum swimming velocity of 150 mm fish at 13°C (Alsop and Wood 1997). Previous studies have shown that training swim protocols result in better swimming performance in critical swimming velocity tests performed the next day (Jain et al. 1997).

Fish then recovered for 60 min at 13±0.3°C before water temperature was increased to the test temperature for each pair of fish (ranging from 13 to 25°C). Water temperature was increased in increments of 1°C 30 min^{-1} and the time that the test temperature was reached was noted, which for the highest test temperature (25°C) was ca. 2400 h. Thus, all fish in the study reached their test temperature at least 8 h before swimming tests began the following morning. Measurements of MR began 30 min after the fish reached the test temperature and continued until 0700 h. The lowest four MR measurements collected during this overnight period were averaged to estimate RMR.

Critical swimming velocity tests at the test temperature began between 0800 and 0900 h to measure MMR for each fish in two phases: a critical swimming velocity test in the first phase followed by a burst swimming test in the second phase. For the critical swimming velocity test, water velocity was again gradually increased until the fish moved off of the chamber floor and began to swim. Once a fish was swimming consistently, water velocity was gradually increased to 30 cm s^{-1} over a 10 min period and then held at 30 cm s^{-1} for 20 min. If a higher initial swimming velocity was required to elicit continual swimming, the fish was held at this initial velocity for 20 min as its first test velocity. Water velocity was then increased in increments of 3 to 6 cm s^{-1} every 20 min until the fish failed to swim continuously. The velocity increment was set to ~10% of the previous test velocity; i.e., if the previous test velocity was between 20 to 39 cm s^{-1}, the velocity increment was 3 cm s^{-1}; when the previous test velocity was between 40 to 49 cm s^{-1}, the velocity increment was 4 cm s^{-1}. Active metabolic rate was monitored at each test velocity by closing the tunnel for two 7- or one 17 min measurement periods after the first 3 min of being flushed with fresh water. Water in the tunnel never dropped below 80% air saturation, which is an oxygen level expected to be considered normoxic. At the end of a measurement period, the next test velocity began with a 3 min flush period. Whenever a fish fell back in the swimming chamber and made full body contact with the downstream screen in the tunnel, water velocity was lowered to 13 to 17 cm s^{-1} for 1 min, and the 20 min timer stopped. After a 1 min recovery, the test velocity was gradually restored over a 2 min period and then the 20 min timer was restarted. Failure velocity was defined when the fish fell back to the downstream screen a second time during the same test velocity. The time of this failure velocity was noted.

For each test velocity, video recordings were observed for quantification of tail beat frequency (TBF measured in Hz). Three 10-s sections of video, where the fish was continuously holding station without contact with the downstream screen, bottom or side of the tunnel were identified. If three replicates were not possible throughout the entire 20 min interval, two replicates were used. If only one replicate was possible, that interval was not quantified. For each of the three (or two) sections, video was slowed to 1/4 to 1/8 of real time speed, and the number of tail beats were counted over 10 s of real time. The three (or two) replicates were then averaged. The same methodology was applied to video recordings taken of fish swimming in the river at temperatures of 14 and 20°C during the study period.

Approximately 50% of the wild fish did not respond as expected to the critical swimming velocity protocol but instead used their caudal fin to prop themselves on the downstream screen to avoid swimming. This behavior was regularly observed at test velocities well above the measured maximum swimming velocity for other fish. Consequently, to estimate MMR for these fish, swimming activity was evoked by rapidly increasing water velocity to a transient velocity stimulus of 70 to 100 cm s^{-1} (increase over 10 s and hold for 30 s or less), then decreasing the velocity back to the test velocity. Fish tended to briefly burst swim off of the downstream screen when velocities exceeded 70 cm s^{-1}. After the transient velocity increase, the fish was allowed to swim without interference (at the test velocity) as long as it continued to swim. For some fish, it was necessary to apply the transient velocity stimulus several times to keep the fish swimming. These fish were otherwise swum identically to fish that swam continuously; i.e., with 20 min test velocity periods and with metabolic rate measurements taken during each test velocity period. Failure for these fish was considered to occur when the fish did not swim upstream to prevent contact with the downstream screen, despite the water velocity being increased to 100 cm s^{-1} and returning to test velocity three times. After a critical swimming velocity trial was terminated, all fish were allowed to recover at velocities of 13 to 17 cm s^{-1} for 20 min.

The subsequent burst swimming test entailed a series of metabolic rate measurements taken at higher, short-duration (30-s) water velocities. To begin the burst swimming test, the water velocity was reset to the initial critical swimming velocity test increment specific to the individual fish—i.e., the first velocity increment at which the fish swam continuously for 20 min. The burst swimming protocol involved swimming a fish at its initial critical swimming velocity test increment for up to 10 min before the water velocity was rapidly increased over ca. 10 s to the maximum speed the fish could swim without contacting the downstream screen and held for ca. 30 s (or less if the fish fell back on to the downstream screen). After the 30-s burst, the velocity was decreased back to the initial critical test velocity for ca. 30 s. This protocol was repeated multiple times for at least 5 min and up to 10 min. Metabolic rate was measured for these fish by flushing the tunnel for the first 3 min of the 10 min continuous swim, then sealing the tunnel for the remaining time. Similarly, the tunnel was flushed for no more than the first

3 min of the 10 min burst swim, and sealed for the remaining time. After completion of the burst swim protocol, fish were allowed at least 60 min of recovery at the test temperature.

Following the 1-h recovery period after the swim tests, water temperature in the tunnels was lowered to ca. 13-15°C over a 30 min period. Fish were then transferred into the individual transport tanks and placed in the flow through holding tanks before measurement and tagging procedures. Fish were anaesthetized for < 5 min with CO_2 (produced by dissolving 2 Alka-Seltzer tablets in 3 l river water) and without losing gill ventilatory movements. The fork length (FL, mm) and mass (g) for each fish was measured, and half duplex PIT (Oregon RFID) tags were placed into the abdominal cavity of the fish through a 1-mm incision through the body wall, just off center of the linea alba. All equipment was sterilized with NOLVASAN S prior to tagging, and wounds were sealed with 3M VetBond. Fish were returned to the transport coolers filled with 13-15°C river water to revive (observed to swim and maintain equilibrium) before being transported to the river capture site for release. At the release site, river water was gradually added to the transport cooler to equilibrate the fish to river water temperature at a rate of 1-2°C h^{-1} before release. Once the acclimated to the river temperature, fish were allowed to swim away volitionally.

To summarize, prior to release back to the river, all fish were subjected to:

- a 1-h adjustment period in the swim tunnel at 13°C;
- a 1-h training swim at 13°C that began at ca. 1600 h;
- a 1-h recovery period at 13°C before the water temperature was warmed to the test temperatures;
- holding at the test temperature for at least 8 h before testing for MMR;
- swimming at various activity levels for minimally 2 h and maximally 6 h until they reached exhaustion;
- a 1-h recovery period at test temperature;
- decrease from test temperature to 13-15°C over 30 min; and
- measurement and tagging.

Data Quality Control, Model Selection and Analyses

Routine metabolic rate quality control (QC) was performed by visually inspecting over night video recordings for fish activity. Data from any fish showing consistent activity over night was discarded. Data from three fish were discarded based on this criterion (W7, W8, and W17). RMR was calculated by averaging the lowest 4 metabolic rate measurements from 30 min after the fish reached the test temperature to 0700 the next morning.
There were two methods of establishing MMR: 1) Swimming (critical swimming velocity and burst performance), and 2) Agitated behavior (i.e., random movements and struggling) in the tunnel. QC criteria for MMR involved assessment of fish behavior in the tunnel via the video, and MR response to incremental increases in tunnel speed. MMR was reported as the single highest MR measurement. The highest MRs observed in this study were concurrent with fish exhibiting intense agitation. For fish not exhibiting intense agitation, the swimming MMR was used as overall MMR. Four of these ‘non-agitated’ fish (W2, W13, W14, and W15) were

discarded due to failure of MR to increase incrementally; despite continuous station-holding swimming with tunnel velocity increases of more than 15 cm s^{-1}.

Four different relationships were examined: 1) RMR versus temperature, 2) MMR versus temperature, 3) AAS versus temperature, and 4) FAS versus temperature. Model fitting was performed in R (http://cran.r-project.org) using the 'lm' function. Confidence intervals and predicted values based on the best-fit model were calculated using the 'predict' function, also in R. To select the model that best described each data set, the r^2 and residuals of each model type were compared. The model with the highest r^2 was chosen, except, when the r^2 of different models were identical, the model with the lowest residual SE was chosen. Four different models were tested: linear, quadratic, antilog base 2, and log base 2 model.

RESULTS

The experimental data table, including raw RMR, MMR, AAS, and FAS data for individual fish are presented in Appendix 4.

1. Routine metabolic rate (RMR) increased exponentially over the range of test temperatures from 13°C to 25°C. This thermal response was fitted with a statistically significant ($P=5.83x10^{-13}$) relationship (Figure 4A), where:

   RMR (mg O_2 $kg^{-0.95}$ min^{-1}) = $5.9513 - 0.5787x + 0.02x^2$

   x = temperature (°C).

   Thus, RMR at 13°C averaged 2.18 ± 0.45 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} and reached 5.37 ± 0.41 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. Consequently, the fish's oxygen demand (cost of basic living) increased by 2.5-fold over the 12°C range for test temperature.

   These results for RMR are consistent with our prediction #1 derived from EPA (2003) criteria and the identical alternative prediction #1. They state that RMR should increase exponentially until the test temperature approaches the upper thermal tolerance limit for *O. mykiss*, which according to published CTmax values is 26°C to 32°C (see Table 1). This prediction could not be further tested because permitting restrictions prevented tests higher than 25°C which is clearly lower than the CTmax because fish survived and even swam for several hours at 25°C.

2. Maximum metabolic rate (MMR) increased linearly with test temperature up to the maximum test temperature of 25°C. This thermal response was fitted with a statistically significant ($P=8.94x10^{-7}$) relationship (Figure 4B), where:

   MMR (mg O_2 $kg^{-0.95}$ min^{-1}) = $1.6359 + 0.3835x$

   x = temperature (°C)

   Thus, MMR at 13°C averaged 6.62 ± 1.03 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1} and increased up to the highest test temperature tested (25°C), where MMR was 11.22 ± 0.86 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1}. Consequently, the maximum oxygen delivery at 25°C was 1.7-times greater than that at 13°C.

   These results for MMR are inconsistent with our prediction #2 derived from EPA (2003) criteria where MMR was expected to peak near to 18°C. Instead, these MMR results are consistent with our alternative prediction #2 that the Tuolumne River population of *O. mykiss* is locally adjusted to warmer temperature, as demonstrated by peak MMR occurring at least 7°C higher than 18°C.

3. Absolute aerobic scope (AAS) was largely independent of test temperature over the range 13-25°C. Indeed, it was only at the two extremes of test temperature that any change in

AAS was statistically discernable. Because of the weak dependence of AAS on test temperature, the best statistical model for these AAS data only approached significance (P=0.06; Figure 4C) where:

AAS (mg O_2 $kg^{-0.95}$ min^{-1}) = -5.7993+1.1263x -0.0265x^2

x = temperature (°C).

This mathematical relationship generated a T_{opt} at 21.2°C with a peak AAS of 6.15 ± 0.71(95% CI) mg O_2 $kg^{-0.95}$ min^{-1}.

These results for AAS are inconsistent with our prediction #3 based on EPA (2003) criteria, but are consistent with our alternative prediction #3 that the Tuolumne River population of *O. mykiss* is locally adjusted by having T_{opt} for AAS that is greater than 18°C i.e., 21.2°C.

4. Contrary to our prediction #4 and our alternative prediction #4, AAS did not significantly decline above the optimal temperature. In fact, the numerical change in average AAS was surprisingly small over the entire test temperature range. Thus, rather than having a well-defined peak to the AAS curve, as expected for fish with a narrow thermal range, as schematically depicted in Figure 1, the results revealed a rather flat curve more similar to one typical of a temperature generalist. Simply, *O. mykiss* in the lower Tuolumne River were able to maintain peak AAS over a wide range of test temperatures well above 18°C. This fact can be best illustrated by two metrics, the thermal range for the statistical 95% CI of AAS and the T_{opt} window for 95% of the peak AAS (i.e., 5.84 mg O_2 $kg^{-0.95}$ min^{-1}).

   The statistical 95% confidence limits for peak AAS extend between 16.4°C and 25°C. Consequently, the numerical decrease in average AAS from 6.15 ± 0.71(95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at T_{opt} to 5.78 ± 1.09(95% CI) mg O_2 $kg^{-0.95}$ min^{-1} at 25°C was only 6% and did not reach statistical significance. Indeed, the AAS measured at 24.5°C (5.89 ± 1.05 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1}) was numerically identical to that measured at 18°C (5.89 ± 0.80 (95% CI) mg O_2 $kg^{-0.95}$ min^{-1}). But when measured at 13°C, AAS was 4.36 ± 1.21(95% CI), which was below the 95% CI for the peak AAS value.
   The numerical 95% peak AAS could be maintained from 17.8°C to 24.6°C, which is a more conservative thermal range for T_{opt}.

5. Although individual variability in FAS was considerable, on average the Tuolumne River population of *O. mykiss* could at least double their RMR across the entire test temperature range from 13 to 25°C. On an individual fish basis, a FAS value exceeding 3.5 was achieved in individual fish tested at 13, 16, and 22°C. Factorial aerobic scope (FAS) declined with temperature. This thermal response was fitted with a statistically significant (P=2.92x10^{-4}) relationship (Figure 4D) where

   FAS = 2.1438 + 0.1744x -0.0070x^2

   x = temperature (°C).

Consequently, the average FAS at 13°C was 3.32 ± 0.41 (95% CI) and decreased to 2.13 ± 0.33 (95% CI) at 25°C. This result is inconsistent with our prediction #5 derived from EPA (2003) criteria , but consistent with our alternative prediction #5 that FAS will remain above a value of 2 at temperatures well above 18°C. Indeed, all individual fish tested up to 23°C had a FAS value >2, with only 4 out of 14 fish tested at 23°C, 24°C and 25°C having a FAS value <2.

6. During swim tests at test temperatures of 14°C and 20°C, a statistically significant linear relationship ($P=2.05 \times 10^{-5}$ for 14°C and 0.009 for 20°C) was determined between MR and Tail Beat Frequency (TBF) (Figure 5).

   For fish tested at 14°C, this relationship was:

   $$MR\ (mg\ O_2\ kg^{-0.95}\ min^{-1}) = 0.75\ (TBF) + 1.05$$

   For fish tested at 20°C, this relationship was:

   $$MR\ (mg\ O_2\ kg^{-0.95}\ min^{-1}) = 1.04\ (TBF) + 1.89$$

   Video analysis of fish in the lower Tuolumne River at 14°C and 20°C revealed for a fish to hold station against a river current required a TBF of 2.94 and 3.40 Hz, respectively. From these TBF values, it was possible using Figure 5 to interpolate a MR associated with *O. mykiss* maintaining station in normal habitat against the Tuolumne River current. These values were 3.26 mg O_2 $kg^{-0.95}$ min^{-1} at 14°C and 5.43 mg O_2 $kg^{-0.95}$ min^{-1} and 20^{o}C. These estimates indicate that the cost of maintaining station increased MR by 1.50- and 2.04-fold, respectively, and used up ca. half of the available FAS (67% and 49%, respectively) at these two temperatures. This meant that the remaining FAS was 2.0 at 14°C and 1.7 at 20°C.

7. After exhaustive exercise, fish quickly recovered their RMR without any visible consequences when they were inspected before being returned to the river. After a 60 min recovery period, MR either had returned to RMR, or was no more than 20% higher than RMR. There were only two exceptions to this generality. Two fish tested at 25°C regurgitated rather large meals of aquatic invertebrates during the recovery from the swim test, and one of these fish died abruptly during the recovery period. No other fish mortality occurred as a result of testing the fish.

   Further evidence of post-release recovery was provided by the six fish that were inadvertently recaptured 1 to 11 days after they had been tested and returned to the river (Figure 3, Appendix 3). All these fish were recaptured in their same habitat unit and within 20 m of the original capture location. All recaptured fish were visually in good condition. Three of these recaptured fish had been tested at one of the highest test temperatures, 23°C.

DISCUSSION

Data Quality

This report contains the first metabolic rate data for the Tuolumne River *O. mykiss* population, which were used to characterize their capacity for aerobic performance over a wide test temperature range that extended above 18°C. The absolute values for RMR and MMR can be compared with the scientific literature even though caution is needed if differences exist in body mass, acclimation temperature, populations and species. For the Tuolumne River *O. mykiss* population RMR increased from 2.18 mg O_2 $kg^{-0.95}$ min^{-1} at 13°C to 5.37 mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. As a generality, a doubling or tripling of RMR is considered a normal biological response for an acute 10°C temperature change (Schmidt-Nielsen 1994). Here, RMR increased by 2.5-times for a 12°C change. By comparison, a study of thermally acclimated and smaller sized (5-7 g) Mount Shasta and Eagle Lake *O. mykiss* found that RMR was similar (2.3-2.8 mg O_2 $kg^{-0.95}$ min^{-1}) at 14°C, but lower (2.9-3.1 mg O_2 $kg^{-0.95}$ min^{-1}) at 25°C (Myrick and Cech 2000, Table 2). Similar RMR values are reported in a wide range of studies for juvenile salmonids (Table 2). Also, when compared with other field-based measurements, but on wild adult salmon (coho, pink and sockeye) at temperatures of 10-16°C (2.9 – 4.3 mg O_2 kg min^{-1}; Farrell et al. 2003), the RMR measured in this study for *O. mykiss* was again lower at these temperatures.

The main methodological challenge with accurately measuring RMR in fish is eliminating spontaneous locomotory activity, which can potentially elevate MR in salmonids more so than any other activity. (Note: An overestimate of RMR reduces the AAS estimate). Therefore, considerable effort was used to select the minimum MR rate measurements to estimate RMR and to use video analysis to confirm that the fish were inactive during the MR measurement, an additional quality control measure that was introduced by Cech (1990). As a result, the variance for RMR of Tuolumne River *O. mykiss* was small despite the fact that the measurements were field-based. The variance was much less than that reported for a field study with adult sockeye salmon (individual RMR values varied by about 2-times) where the experimental protocol was limited to only one RMR measurement (Lee et al. 2003). As a result of this low variance, the statistical model explained 80% of the variance in RMR. Therefore, we are confident in the RMR measurements generated for this report.

Normally, RMR is measured in a post-absorptive state (i.e., following a period of starvation for ca. 24h) because the digestive process is an activity that requires an increase in RMR (Jobling 1981). In the present study, however, the digestive state of the wild fish could not be controlled because it would take a day or longer to fully digest a meal and return to a post-absorptive state (Jobling 1981). In fact, feces were regularly found in the swim tunnels after the overnight acclimation period, which indicated that fish in the river were feeding and that the digestive process had continued for at least part of the overnight period. Therefore, although the present measurement of RMR could have been elevated by a variable contribution for digestion, our RMR values still agree with or fall below comparable literature values, suggesting that digestion was not a major contributor to the RMR values measured here. Nevertheless, we cannot be certain that we measured standard metabolic rate (SMR), which is more typically used in traditional laboratory experiments to assess AAS. SMR would be lower than RMR, which

would result in an underestimate of AAS and FAS when compared with literature that used SMR for these estimates.
The methodological challenge with accurately measuring MMR in wild fish is that fish vary in their willingness to participate in forced activity because they are naive to the holding conditions and to the actual swim challenge. Thus, while it is impossible to overestimate MMR and AAS, MMR and AAS can be underestimated if a fish chooses not to swim maximally. While it is possible that MMR and therefore AAS were underestimated in this field study, we gave the wild fish a training swim and then used four different testing protocols to generate a MMR measurement to minimize this complication. Indeed, because some of the wild Tuolumne River *O. mykiss* were reluctant to perform a U_{crit} protocol, a burst swimming protocol was used to generate MMR. The four protocols were:

1. continuous swimming with incremental increases in velocity;
2. a combination of continuous swimming and short velocity bursts to push fish off of the downstream screen;
3. a 10 min burst protocol of alternating 30 s of a very high velocity burst with 30 s of low velocity burst (aimed at maintaining moderate swimming); and
4. spontaneous intense activity during RMR measurements (rarely used, but sometimes MR was greater than the for other 3 protocols).

For Tuolumne River *O. mykiss*, the linear regression of MMR versus temperature estimates that MMR at 13°C was 6.62 mg O_2 $kg^{-0.95}$ min^{-1} and increased to 11.22 mg O_2 $kg^{-0.95}$ min^{-1} at 25°C. The statistical model for MMR explained 50% of the individual variance for the *O. mykiss* tested. We are unaware of any data in the literature assessing the response of MMR to warming in juvenile *O. mykiss*, but the average MMR value 7.4 mg O_2 $kg^{-0.95}$ min^{-1} here at 15°C is at the high end of the range (2.9 to 8.3 mg O_2 $kg^{-0.95}$ min^{-1}) reported in the literature for smaller (2-13 g) *O. mykiss* (Table 2). Also at 15°C, we found an average AAS of 5.1 mg O_2 $kg^{-0.95}$ min^{-1} and FAS of 3.2, both of which were on the high end of the range of reported values in the literature (1.8-5.8 mg O_2 $kg^{-0.95}$ min^{-1} and 2.2-5.8, respectively, Table 2). When compared with similar field measurements on wild adult salmon (coho, pink and sockeye) at temperatures of 10-16°C (8.6-12.6 mg O_2 kg min^{-1}; Farrell et al. 2003), the MMRs measured here overlap with the lower end of this range. The individual variation for MMR was greater than that for RMR in Tuolumne River *O. mykiss*, but less than the individual variation reported for MMR values in a field study of adult sockeye salmon (Lee et al. 2003). It is interesting that the variation in MMR correlated with behavior, such that the fish that displayed frequent spontaneous activity during RMR and U_{crit} tests had the highest MMR within a temperature group. Fish that swam continuously throughout a U_{crit} test without many extra stimuli to encourage swimming invariably had the next highest MMR. The lowest MMR was for fish that propped themselves with their caudal fin to avoid swimming despite repeated stimuli with short velocity bursts and this behavior may have prevented a proper estimate of MMR.

Reaction norms defined by the shape of the response curves in Figure 4 allow for proper mathematical and statistical consideration of the thermal range of performance, a concept that is fully endorsed by EPA (i.e., the 7DADM designation "*recognizes the fact that salmon and trout juveniles will use waters that have a higher temperature than their optimal thermal range.*"). Indeed, given the rather flat reaction norm centered around a T_{opt} of 21.2°C shown here for the

Tuolumne River *O. mykiss*, it is certainly more appropriate to talk about a thermal range of performance. Thus, given the good agreement with existing literature for MR measurements combined with the fact that the shape of the response curves will be independent of the methodological concerns noted above, we are confident in using these response curves to test the predictions based on EPA (2003) and our alternative predictions.

Evidence for Local Thermal Adjustment

Our predictions based on EPA (2003), as listed above, assumed that the Tuolumne River *O. mykiss* population would perform similarly to Pacific Northwest *O. mykiss* populations used to set the 7DADM by EPA (2003). Our alternative predictions, however, allow for the possibility of local thermal adjustment to a warmer river habitat. Collectively, the results show clear deviations from our predictions based on EPA (2003), and consistency with the alternative predictions, which suggests the likelihood that the Tuolumne River *O. mykiss* population is locally adjusted to warm thermal conditions. In particular, the T_{opt} for AAS was 21.2°C, markedly higher than 18°C. Furthermore, AAS at 18°C was numerically the same as that at 24.5°C. Therefore, we discovered that the Tuolumne River *O. mykiss* population has a wide thermal range for optimal performance. Indeed, one fish was inadvertently recaptured in good visual condition from its original habitat location in the Tuolumne River 11 days after being tested at 23°C for 14 h and performing demanding swim tests. However, given that the CTmax could not be determined in the present work and that MMR increased up to the highest test temperature (25°C), it was impossible to determine the upper thermal limit when MMR collapses, which means that alternate metrics must be used to set the upper thermal limit for the Tuolumne River *O. mykiss* population.

The present work provides three useful metrics of the optimal temperature range. Using the T_{opt} of 21.2°C for the mathematical peak of AAS, the least conservative metric is the thermal range for the 95% CI of peak AAS, which is 16.4°C and 25°C. The next metric, which was nearly as conservative as the first, is the thermal range where AAS remained within 5% of the peak AAS at 21.2°C, which is 17.8°C to 24.6°C for Tuolumne River *O. mykiss*. The small difference between these two temperature ranges is more a result of the individual variation in the data. The third and most conservative metric defines temperatures where FAS values for all fish were >2, which would dictate a thermal optima range from 13 to 22°C, although the average FAS value was 2.13 at 25°C. Thus, the performance of the Tuolumne River *O. mykiss* population remained sufficiently elevated well beyond 18°C, which is compelling evidence of local adjustment to warm conditions.

Yet, there were important indications that a small percentage of individuals were taxed at temperatures of 23-25°C by the thermal testing and intensive swim imposed on them outside of their normal habitat over a 24-h period. Such individual variability in upper thermal performance is not unexpected. Indeed, Hokanson et al. (1977) reported heightened mortality only during the initial 20 days of a growth trial for *O. mykiss* at supra-optimal temperatures. In the present study, the telltale signs were that 4 out of 13 individuals tested at 23-25°C had a FAS < 2. In the next section, we suggest that fish need a FAS value of ca. 2 for proper digestion. Interestingly, two fish regurgitated their stomach contents at 25°C, a symptom common during extreme athletic exertion in humans when metabolic rate over-taxes oxygen supply. Lastly, the only fish mortality occurred in the recovery period (a phenomenon known as 'delayed mortality') after one fish was tested at 25°C.

Ecological Relevance of the Present Findings

Establishing ecological relevance of physiological data, such as those collected for the present report, has always been a challenge because of the multiple factors that influence fish distributions, behaviors and performance. Here, we measured the aerobic capacity of the Tuolumne River *O. mykiss* population in a field setting to improve the ecological relevance by minimizing fish transport and handling. After a rapid recovery from our exhaustive swim and thermal tests (as seen in MR measurements during 60 min recovery from swimming tests), the test fish appeared to reestablish their original habitat in the Tuolumne River because a portion of the test fish were inadvertently recaptured in the river within 20 m of the original capture site. This excellent recovery behavior from intense testing seemed to be independent of the test temperature because fish were recaptured after a wide range of test temperatures (16-23°C; see Appendix 3)

To provide ecological relevance to physiological findings some 60 years ago, Fry (1947) introduced the concepts of a fish being metabolically loaded and metabolically limited to explain environmental effects on fishes. Simply put, a metabolic load from an environmental factor increases the oxygen cost of living (e.g., it costs energy to detoxify a poison, or, as in the present study case, a thermal increase in RMR). Conversely, a metabolic limit from an environmental factor decreases the MMR, leaving less oxygen available for activities. More broadly, the allocation of energy and tradeoffs is now a fundamental tenant of ecological physiology, especially in fishes (see review by Sokolova et al. 2012). Like all other temperature studies with fish, we found that RMR increased between 13 and 25°C, but there was nothing untoward in the magnitude of this thermal response (a 2.5-times increase in RMR over this temperature range).

MMR increased with temperature from 13 to 25°C, which would mean that as fish encounter higher temperatures, they have the capacity to perform an activity at a higher absolute rate, i.e., swim faster to capture food or avoid predators, digest meals faster, detoxify chemicals faster, etc. They certainly swam harder with temperature in the present study. Thus, the Tuolumne River *O. mykiss* population can perform at a higher capacity level at 25°C compared with either 13°C or 18°C. The T_{opt} for AAS occurred at 21.2°C, which is the temperature that the Tuolumne River *O. mykiss* population is predicted to have its highest absolute capacity for aerobic activity.

However, MMR increased by 1.7-times versus 2.5-times for RMR between 13°C and 25°C. Consequently, FAS decreased with temperature. The FAS value measures the capacity for a proportional increase in RMR and typically decreases with temperature in fishes (Clark et al. 2011). Thus, the present finding for FAS was not unexpected. Moreover, being able to maintain FAS above 2 (i.e., being able to at least double its RMR) may have ecological relevance to fish (FAS = 2) for two important activities: digesting a full stomach and maintaining station in a flowing river.

Many laboratory studies with fish have examined the metabolic cost of digesting a full stomach. The peak oxygen cost of digesting a meal increases with temperature and meal size, but peak

MR does not increase by more than 2-fold at the temperatures used here and with a meal size typical for a salmonid (e.g., Jobling 1981; Alsop and Wood 1997; Fu et al. 2005; Luo and Xie 2008). Therefore, a FAS value of 2 can be used as an index that a fish has the aerobic capacity to digest a full meal, and all individual fish achieved this performance up to 23°C. As a result of high temperature, a fish would digest the same meal with a similar overall oxygen cost but at a faster rate. This means that the fish could eat more frequently and potentially grow faster at a higher temperature with a FAS >2. Thus, the important ecological consideration is whether or not there is sufficient food in the Tuolumne River to support the highest MR associated with high temperature. All available studies suggest that the Tuolumne River population is not food limited, including direct studies of Tuolumne River Chinook salmon diet (TID/MID 1992, Appendix 16), long-term benthic macro-invertebrate sampling data collected from 1988–2008 (e.g., TID/MID 1997, Report 1996-4; TID/MID 2009, Report 2008-7), as well as the relatively high length-at-age for *O. mykiss* sampled in 2012 (Stillwater Sciences 2013). Indeed, the *O. mykiss* sampled for the current study were apparently feeding well in the river during summer months given the high condition factors (see Appendix 2), feces being regularly found in the swim tunnel and two test fish regurgitating rather large meals post-exhaustion.

Here, we took advantage of the video analysis of the swimming behaviors of individual *O. mykiss* in the Tuolumne River habitat to provide a second evaluation of the ecological relevance of MR data. This analysis revealed a common set of swimming behaviors that *O. mykiss* used to maintain station in the water current, as well as darting behaviors used either to protect their territory or to grab food floating down the river. Because maintaining station against a water current requires a sustained swimming activity that is functionally analogous to steady swimming at one of the velocity increments in the swim tunnel, it was possible to estimate the tail beat frequency (TBF) while performing this normal river activity. Then, using Figure 5, the TBF for station holding was compared with the TBF used while swimming in the swim tunnel to determine a MR. Thus, the estimated oxygen cost of maintaining station in the Tuolumne River by *O. mykiss* at 14°C was found to increase metabolism to 1.5-times RMR, leaving fish with a FAS of 2, and therefore plenty of aerobic scope for additional activities besides maintaining station. Similarly at 20°C, maintaining station increased metabolic rate to 2-times RMR, and the remaining FAS was 1.7. Therefore, by combining laboratory and field observations, we can conclude that the Tuolumne River *O. mykiss* population at 20°C have an aerobic capacity to easily maintain station in their normal river habitat and additionally nearly double their RMR for other activities, or relocating to a lower water flow area to perform other activities.

According to Issue Paper 5 (EPA 2001)"*Acclimation is different from adaptation. Adaptation is the evolutionary process leading to genetic changes that produce modifications in morphology, physiology, and so on. Acclimation is a short-term change in physiological readiness to confront daily shifts in environmental conditions. The extent of the ability to tolerate environmental conditions (e.g., water temperature extremes) is limited by evolutionary adaptations, and within these constraints is further modified by acclimation*." Here we did not evaluate the possibility that the Tuolumne River *O. mykiss* population can thermally acclimate to warmer river temperatures as the summer progresses, due to the available sample of a maximum of 50 individuals and their habitat temperature. Since, the instantaneous temperature in the habitat where the test fish were captured was between 12.7 and 17.1°C (see Appendix 1), the upper thermal performance determined here may underestimate thermal performance if the Tuolumne River *O. mykiss* can acclimate to a higher river temperature. In this regard, the study of the thermal physiology of Mount Shasta and Eagle Lake *O. mykiss* (Myrick and Cech 2000) after thermal acclimation is particularly informative. Growth rate of the Mount Shasta strain was fastest at acclimation temperatures of 19 and 22°C, temperatures that bracket the T_{opt} for AAS determined here for Tuolumne River *O. mykiss*. However, growth of the Mount Shasta strain stopped at 25°C, which is consistent with our result that FAS approached 2 at 25°C. In contrast, growth rate for the Eagle Lake strain was fastest at 19°C and decreased at 22°C. The Eagle Lake strain actually lost weight at 25°C, which indicated that food intake was not keeping pace with the energy requirements to sustain the RMR at this temperature, perhaps because of a limitation on AAS. Thus, the Mount Shasta strain of *O. mykiss* was better able to thermally acclimate to temperatures above 20°C than the Eagle Lake strain. The potential for thermal acclimation in Tuolumne River *O. mykiss* is unstudied.

With clear evidence that California strains of *O. mykiss* grow optimally at acclimation temperatures >18°C and that local differences among strains amount to as much as a 3°C shift in the optimum temperature for growth, there is a precedent that the thermal range for optimal performance can reach 22°C for local populations of *O. mykiss*. Indeed, the new data presented here adds to this evidence of local adjustments of *O. mykiss* to warm river habitats, because while T_{opt} for AAS was 21.2°C, AAS remained within 5% of the peak AAS up to 24.6°C and all fish maintained a FAS value >2 up to 23°C.

CONCLUSION

High quality field data were generated on the physiological performance of Tuolumne River *O. mykiss* acutely exposed to a temperature range of 13 to 25°C. These data on the RMR, MMR, AAS, and FAS were consistent with higher thermal performance in Tuolumne River *O. mykiss* compared to that used to generate the 7DADM value of 18°C using Pacific northwest *O. mykiss* (EPA 2003). These new data are consistent with recent peer-reviewed literature that points to local thermal adjustments among salmonid populations. Therefore, these data provide sound evidence to establish alternative numeric criteria that would apply to the Tuolumne River *O. mykiss* population below La Grange Diversion Dam. Given a measured T_{opt} for AAS of 21.2°C, and that the average AAS remained within 5% of this peak performance up to 24.6°C, and all fish maintained a FAS value >2 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, be used to determine a 7DADM value for this population.

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FIGURES

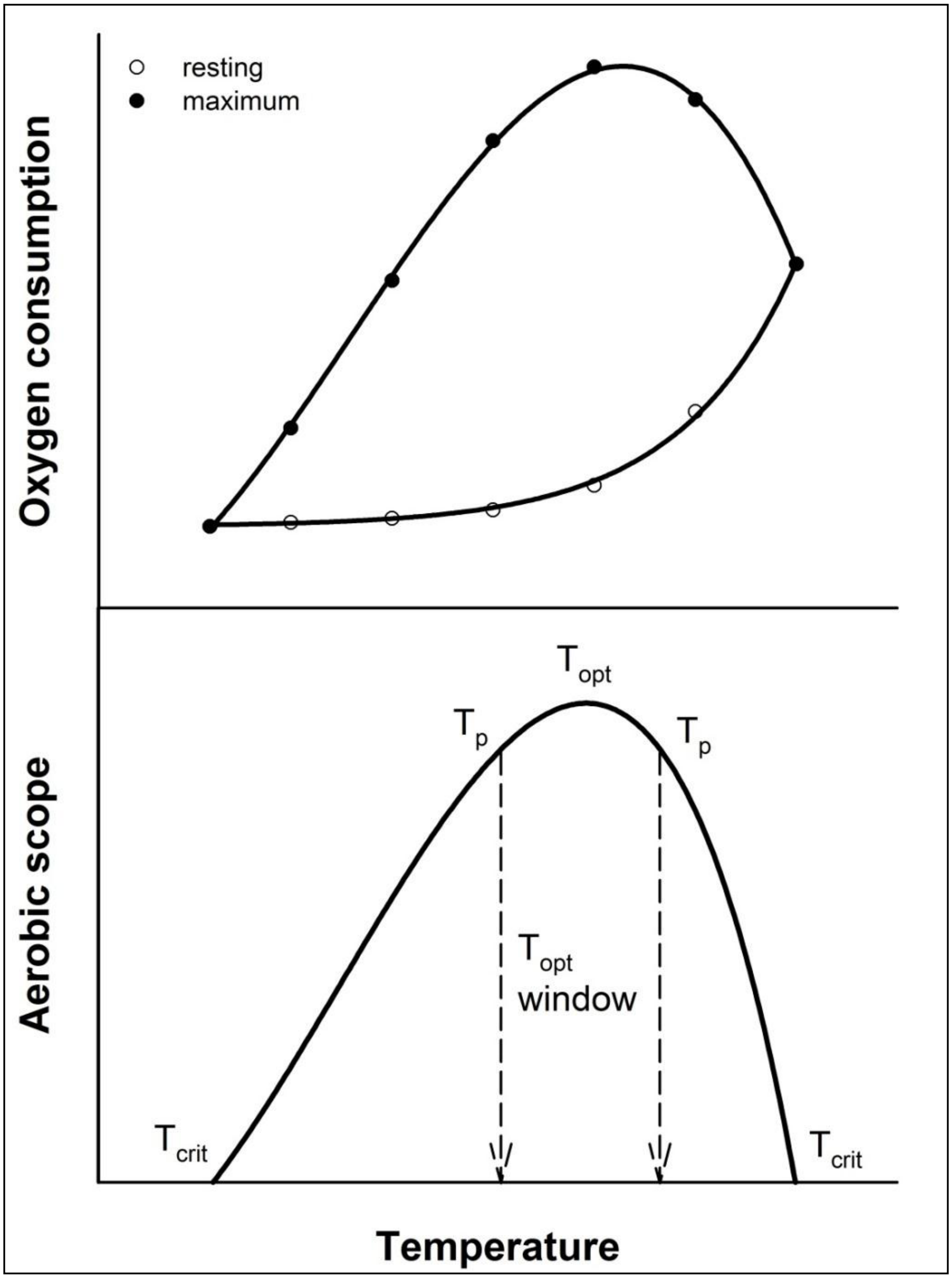
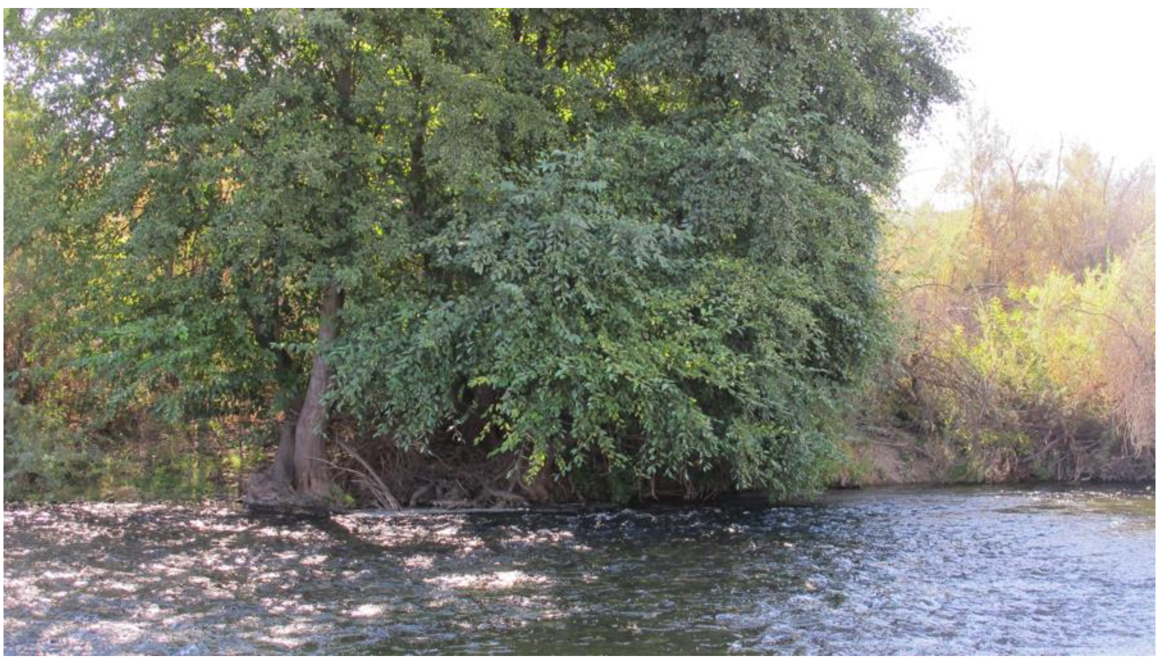


Figure 1. **Schematic representation of the resting metabolic rate (= routine; RMR) and maximum metabolic rate (MMR) and aerobic scope (AS = MMR-RMR) for a temperature specialist. See text for details. T_{opt} = optimum temperature, T_p = pejus temperatures which set the thermal window or range in which 95% of the peak value for AS can be maintained; T_{crit} = critical temperatures where there is no aerobic scope. (Parsons 2011).**

(a) RM 51.6

(b) RM 50.7

(c) RM 50.4



(d) RM 49.1

Figure 2. Representative photographic images (a-d) of the four capture locations for Tuolumne River *O. mykiss*, by river mile.

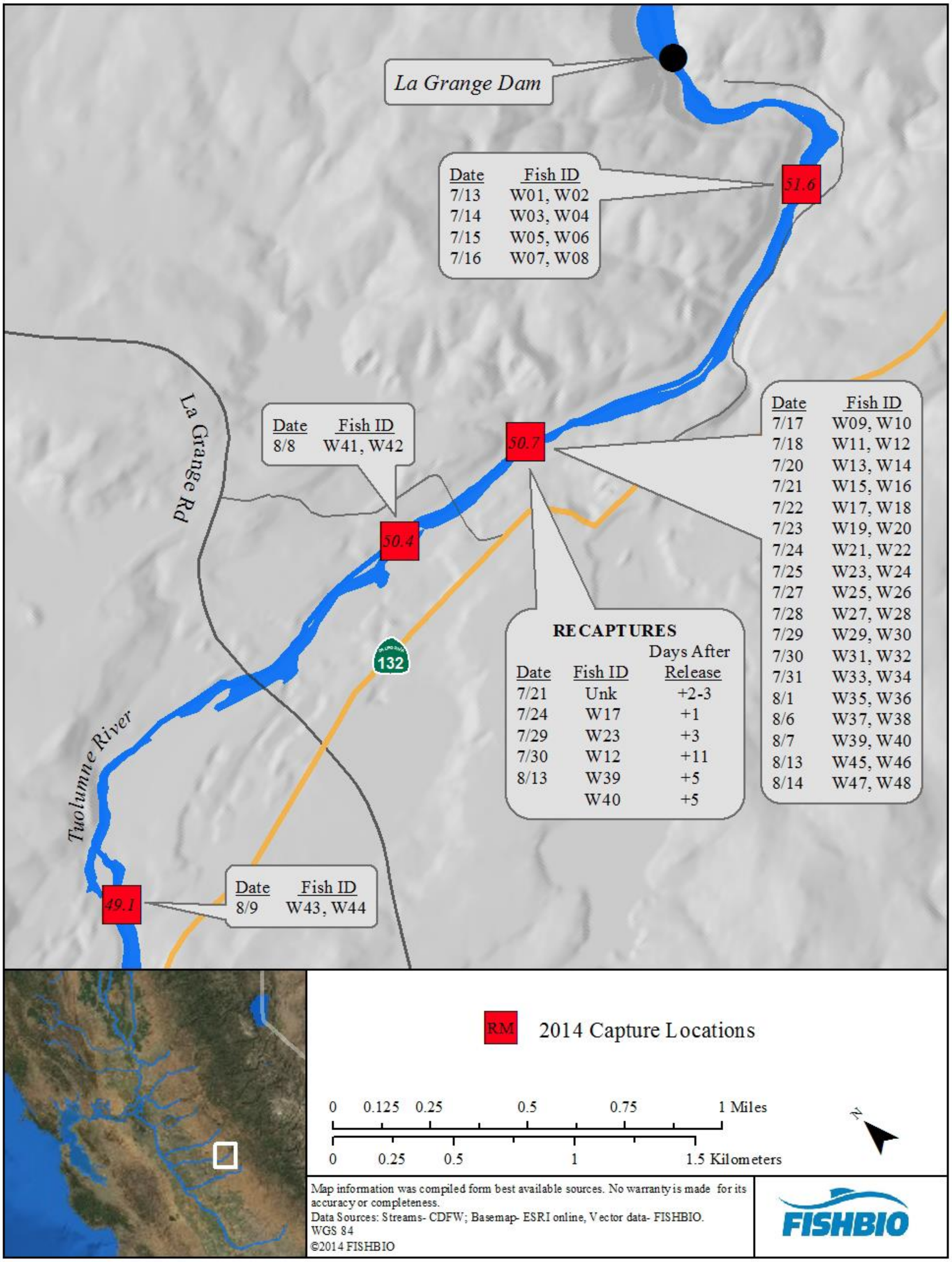


Figure 3. Map of capture and recapture locations of all Tuolumne River *O. mykiss* test fish.

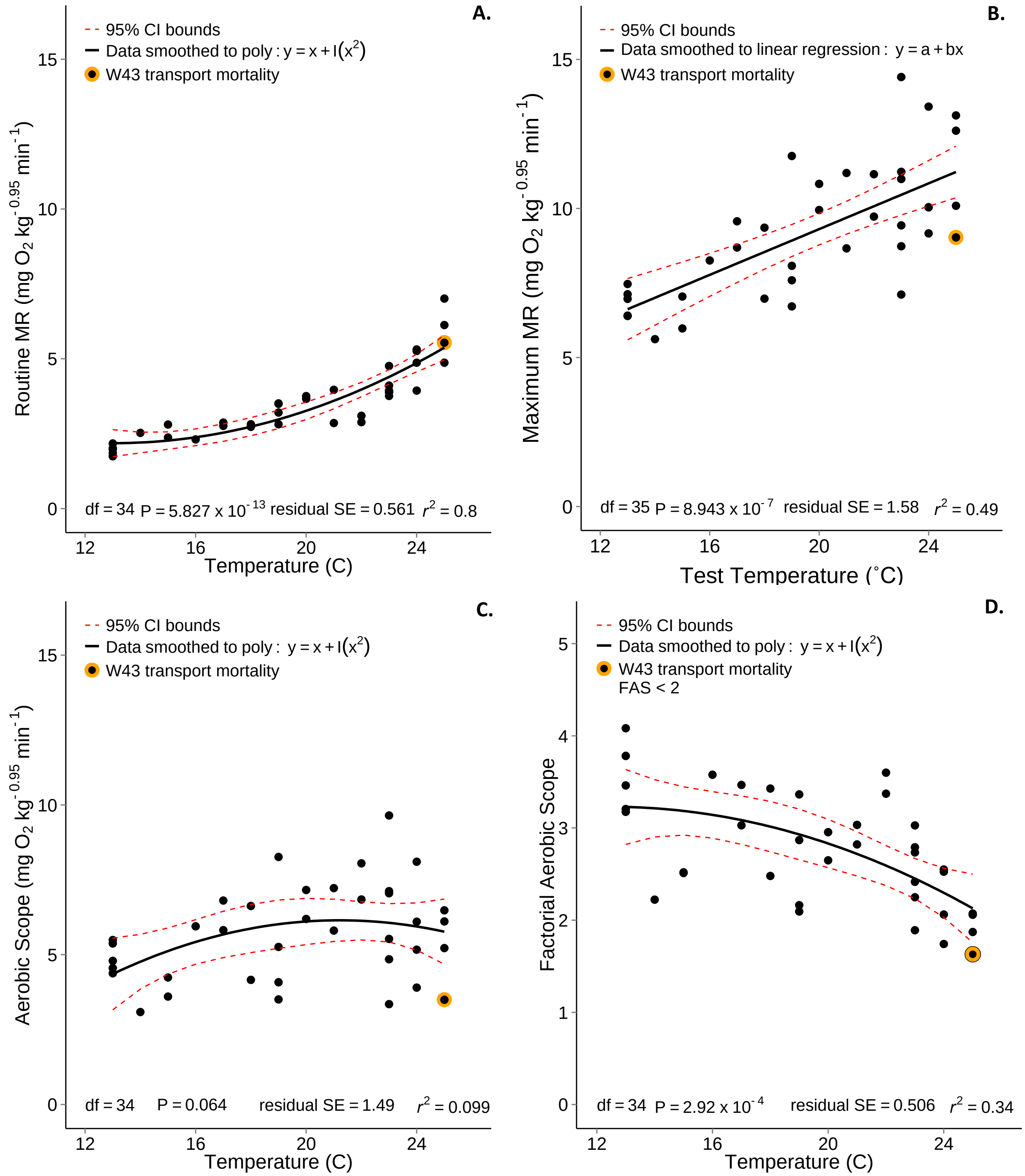


Figure 4. **The relationships between test temperature and the routine (RMR) and maximum metabolic rate (MMR) of Tuolumne River *O. mykiss*. Absolute aerobic scope (AAS) and factorial aerobic scope (FAS) were derived from the MR measurements. Each data point represents an individual fish tested at one temperature. These data were given a best-fit mathematical model (solid line or curve) and the 95% confidence intervals for this line are indicated by the broken lines.**

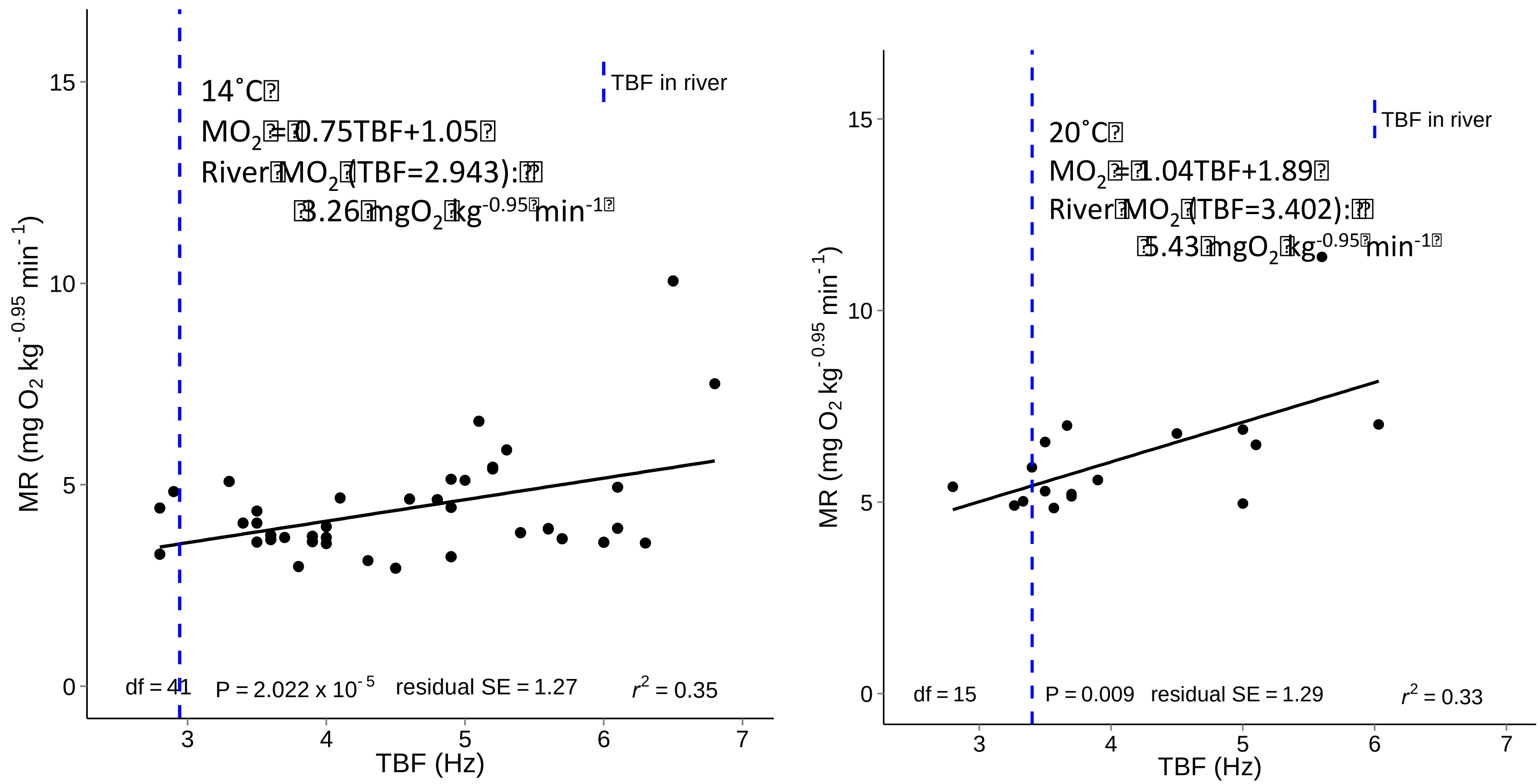


Figure 5. **The relationship between tail beat frequency (TBF; Hz) and metabolic rate (MR; mg O_2 kg$^{-0.95}$ min^{-1}) measured when Tuolumne River *O. mykiss* were swimming continuously in a swim tunnel at 14 °C or 20°C. The solid black line represents the linear regression based on the data for N=7 fish at 14°C and N=5 fish at 20oC). The blue dashed lines represent the estimated TBF (2.94 Hz at 14°C and 3.40 Hz at 20°C (bottom graph) taken from videos of *O. mykiss* maintaining station in a water current in their normal Tuolumne River habitat.**

TABLES

Table 1. Literature values of critical thermal maximum (CTmax) for *O. mykiss* populations.

Acclimation Temperature (°C)	CTmax (°C)	Heating rate (°C min-1)	Mass (g)	Length (cm)	Strain Source	Reference
8	26.9 ± 0.12	0.1		11 – 18	Washington	Becker and Wolford 1980
9.8	27.9 ± 0.05	0.3		15,3 ± 0.25	Pennsylvania	Carline and Machung 2001
10	28.5 ± 0.28	0.02				Lee and Rinne 1980
10	28.0 ± 0.12	0.3	~15	~10	Missouri	Currie et al. 1998
10	27.7 ±0.08	0.3	12.9 ± 0.6		California	Myrick and Cech 2000
11	27.5	0.3	8.0 ± 1.6		California	Myrick and Cech 2005
11 *	29.0 ± 0.05	0.3	2.4 ± 0.5		British Columbia	Scott 2012
13	27.9 ±0.14	0.33		21.8±0.4	Ontario	Leblanc et al. 2011
14	28.5±0.11	0.3	13.8 ± 0.8		California	Myrick and Cech 2000
14	29.4 ±0.1	0.03%	41 - 140		Oregon	Rodnick et al. 2004
15	29.4 ± 0.08	0.3				Strange et al. 1993
15	29.1 ± 0.09	0.3	~15	~10	Missouri	Currie et al. 1998
15	27.7 ±0.03	0.0014 #	89.9 ± 5.4	11.9 – 0.3	North Carolina	Galbreath et al. 2006
15	28.4	0.3	9.3 ± 2.0		California	Myrick and Cech 2005
15	~29.65	0.083 &			Miyazaki, Japan	Ineno et al. 2005
15	29.0 ± 0.02	0.3/0.1	30.2 ± 0.3	13.0 ± 0.4	Western Australia	Present study
18	31.2	0.3		4.1 – 20	Arizona	Recsetar et al. 2012
19	29.6	0.3	14.3 ± 2.9		California	Myrick and Cech 2005
19	29.9 ± 0.17	0.3	11.8 ± 0.7		California	Myrick and Cech 2000
20	29.35 ± 0.19	0.02				Lee and Rinne 1980
20	29.8 ±0.12	0.3	~2	~4	Missouri	Currie et al. 1998
20	~30.4	0.083 &			Miyazaki, Japan	Ineno et al. 2005
22	30.9 ± 0.13	0.3	9.29 - 0.99		California	Myrick and Cech 2000
25	31.75 ± 0.1	0.3	6.1 - 0.63		California	Myrick and Cech 2000

*fish held at 10 ~12°C.

& temperature was increased at 5°C h^{-1}.

% temperature was increased at 2°C h^{-1}.

temperature was increased at 2C day^{-1}.

Table 2. Literature values for routine metabolic rate (RMcR), maximum metabolic rate (MMR), absolute aerobic scope (AAS) and factorial aerobic scope (FAS) of juvenile salmonid fishes.

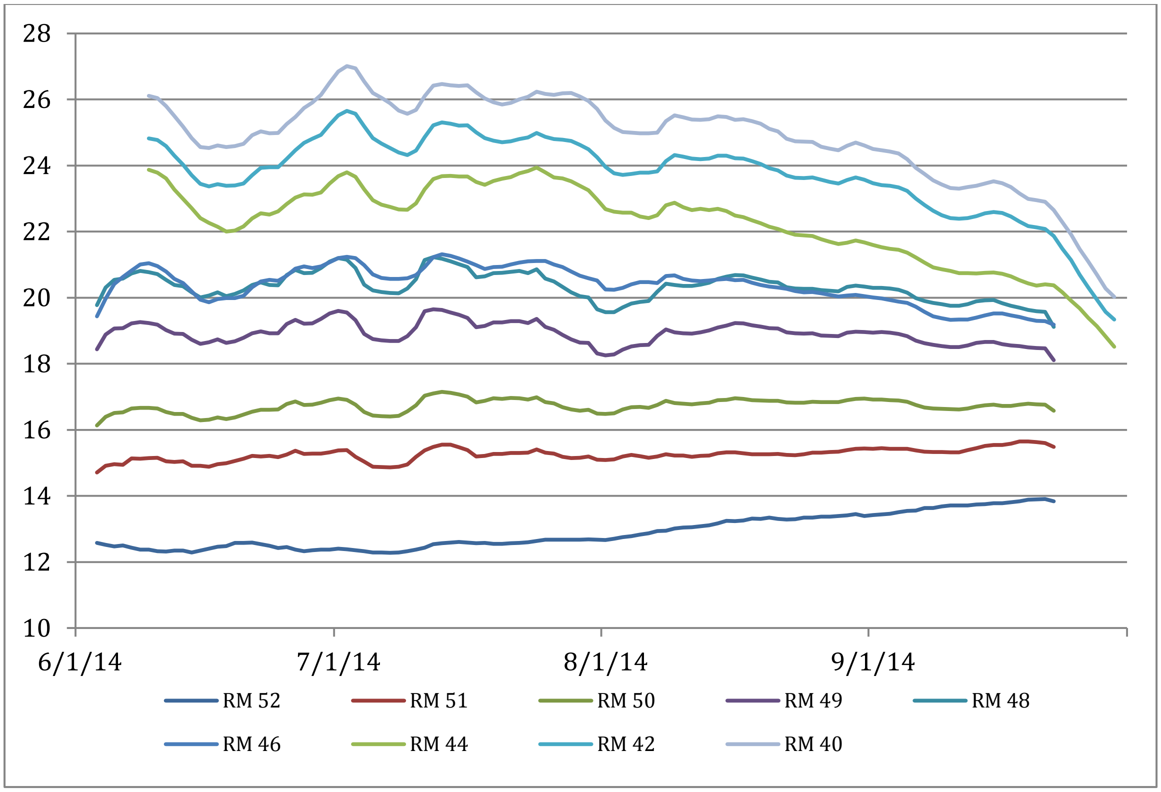
Species	Source[1] (test location)	Mass (g)	Temperature (°C)		Metabolic rates (mg O_2 $kg^{-0.95}$ min^{-1})			FAS	Reference
			Acclimate	Test	RMR	MMR	AAS		
Rainbow trout	Hatchery (L)	13	15	15	0.5	2.9	2.4	5.8	Alsop and Wood 1997
	Hatchery (L)	6	15	15	1	2.8	1.8	2.8	Alsop and Wood 1997
	Hatchery (L)	2-3	15	15	3.9	8.7	4.8	2.2	Scarabello et al. 1991
	Hatchery (L)	6	15	15	2.5	8.3	5.8	3.3	Scarabello et al. 1992
	Hatchery (L)	18	17	17	3.9	7	3.1	1.8	McGeer et al. 2000
	Eagle Lake Wild[2] (L)	6.9	10	10	2.6				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	7.2	14	14	2.8				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	14.1	19	19	2.6				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	13.4	22	22	2.9				Myrick and Cech 2000
	Eagle Lake Wild[2] (L)	5	25	25	3.1				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	10	10	10	2				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	7.5	14	14	2.3				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	24.5	19	19	2.2				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	15	22	22	2.4				Myrick and Cech 2000
	Mt. Shasta Wild[2] (L)	5.4	25	25	2.9				Myrick and Cech 2000
Steelhead trout	Wild (H/F)	1.7	8.3	8.3	1.8-3.4	5.7-9.1			Van Leeuwen et al. 2011
	Hatchery (H/F)	3.3	12.3	12.3	1.9-3.6	5.5-9.7			Van Leeuwen et al. 2011
Rainbow trout	Wild[2] (L) (territorial)	60-80		13	0.6-1.9				Sloat and Reeves 2014
	Wild[2] (L) (dispersing)	60-80		13	0.6-1.5				Sloat and Reeves 2014
Rainbow cutthroat hybrid	Hatchery (F)	20-70	9.5-11	9.5-11	2.3				Rasmussen et al. 2012
Cutthroat trout	West slope Wild (F)	20-100	9.5-11	9.5-11	2.6				Rasmussen et al. 2012
Redband trout	Wild Bridge Creek (F)	92 (150-200 mm)	12-24*	13	1.8	8.5	6.7	4.7	Gamperl et al. 2002
	Wild Bridge Creek (F)	108 (150-200 mm)	12-24*	24	4.5	14	9.5	3.1	Gamperl et al. 2002
	Wild Little Blitzen River (F)	58	12-18*	13	2.4	12	9.6	5.0	Gamperl et al. 2002
	Wild Little Blitzen River (F)	71	12-18*	24	5.6	14	8.4	2.5	Gamperl et al. 2002

Species	Source[1] (test location)	Mass (g)	Temperature (°C)		Metabolic rates (mg O_2 $kg^{-0.95}$ min^{-1})			FAS	Reference
			Acclimate	Test	RMR	MMR	AAS		
	Wild 12 Mile Creek (F)	56	19-30 (23.4)*	24	4.7	18.3	13.6	3.9	Rodnick et al. 2004
	Wild Rock Creek (F)	50	12-27 (18.7)*	24	4.7	18	13.3	3.8	Rodnick et al. 2004
	Wild Bridge Creek (F)	63	13-21 (17)*	24	4.6	15.6	11	3.4	Rodnick et al. 2004
Sockeye salmon	Wild (L)	37 (170 mm)	5	5	0.9	7.6	6.7	8.4	Brett 1964
	Wild (L)	33(160 mm)	10	10	1.4	8.7	7.3	6.2	Brett 1964
	Wild (L)	55 (190 mm)	15	15	1.7	14.2	12.5	8.4	Brett 1964
	Wild (L)	63 (190 mm)	20	20	2.1	13.1	11	6.2	Brett 1964
	Wild (L)	52 (180 mm)	24	24	0.8	12.7	11.9	15.9	Brett 1964
	Wild (L)	20-60	5.3	5.3	0.5	6.9	6.4	13.8	Brett and Glass 1973
	Wild (L)	19-60	15	15	0.9	9.9	9	11.0	Brett and Glass 1973
	Wild (L)	20-50	20	20	1.7	12.5	10.8	7.4	Brett and Glass 1973
Coho salmon	Wild (H/F)	3.9	8.3	8.3	1.5-3.1	3.6-6.2			Van Leeuwen et al. 2011
	Hatchery (H/F)	5.4	12.3	12.3	1.1-2.3	3.8-6.5			Van Leeuwen et al. 2011
	Wild[2] (F)	40-100	9.5-11	9.5-11	3.2				Rasmussen et al. 2012
	Wild (L)	4.3	14	14	1.6				Van Leeuwen et al. 2012
Redband trout	Wild 12 Mile Creek (F)	94	19-30 (23)*	14	1.6				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	24	2.3				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	26	4.8				Rodnick et al. 2004
	Wild 12 Mile Creek (F)	94	19-30 (23)*	28	5.6				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	14	1.8				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	24	3.7				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	26	5.7				Rodnick et al. 2004
	Wild Rock Creek (F)	54	12-27 (19)*	28	6.1				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	14	2.3				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	24	4.2				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	26	5.6				Rodnick et al. 2004
	Wild Bridge Creek (F)	79	13-21 (17)*	28	6.7				Rodnick et al. 2004

[1] L = laboratory; H = hatchery; F=Field.
[2] Spawned in a hatchery.
*Acclimations to cycled temperature regime of range indicated, and average in brackets if reported.

APPENDICES

Appendix 1. Tuolumne River 7-day average of maximum daily temperatures (7DADM) from June 1 to September 30, 2014. Thermograph data provided by TID (Patrick Maloney).



Appendix 2. Capture release table. Fish capture and release locations and physical conditions.

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W01	N - 37.66574 W - 120.44421	7/13	9:45	12.9	N - 37.66574 W - 120.44421	7/14	15:35	14.4	4 FW Riffle (side channel #3)	51.5	
W02	N - 37.66574 W - 120.44421	7/13	11:24	13.2	N - 37.66574 W - 120.44421	7/14	15:36	14.4	4 FW Riffle (side channel #3)	51.5	
W03	N - 37.66532 W - 120.44482	7/14	11:04	13.5	N - 37.66518 W - 120.44509	7/15	17:25	14.1	4 FW Riffle (side channel #3)	51.5	
W04	N - 37.66538 W - 120.44470	7/14	11:08	13.5	N - 37.66518 W - 120.44509	7/15	17:25	14.1	4 FW Riffle (side channel #3)	51.5	
W05	N - 37.66524 W - 120.44424	7/15	9:50	12.8	N - 37.66544 W - 120.44449	7/16	13:07	14.6	4 FW Riffle (side channel #3)	51.5	
W06	N - 37.66536 W - 120.44474	7/15	10:53	12.9	N - 37.66544 W - 120.44449	7/16	12:00	13.4	4 FW Riffle (side channel #3)	51.5	Fish not measured or PIT tagged to limit handling
W07	N - 37.66544 W - 120.44449	7/16	9:52	12.9	N - 37.66510 W - 120.44515	7/17	13:16	14	4 FW Riffle (side channel #3)	51.5	
W08	N - 37.66544 W - 120.44449	7/16	10:10	12.7	N - 37.66510 W - 120.44515	7/17	13:16	14	4 FW Riffle (side channel #3)	51.5	
W09	N - 37.66586 W - 120.45826	7/17	9:10	13.5	N - 37.66581 W - 120.45829	7/18	14:36	16	11 FW Riffle	50.7	
W10	N - 37.66586 W - 120.45826	7/17	9:24	13.5	N - 37.66581 W - 120.45829	7/18	14:36	16	11 FW Riffle	50.7	
W11	N - 37.66581 W - 120.45829	7/18	8:40	13.7	N - 37.66581 W - 120.45829	7/19	14:49	15.5	11 FW Riffle	50.7	
W12	N - 37.66581 W - 120.45829	7/18	8:40	13.7	N - 37.66581 W - 120.45829	7/19	14:49	15.5	11 FW Riffle	50.7	
W13	N - 37.66579 W - 120.45832	7/20	8:48	13.4	N - 37.66585 W - 120.45823	7/21	13:59	15.3	11 FW Riffle	50.7	
W14	N - 37.66579 W - 120.45832	7/20	8:48	13.4	N - 37.66585 W - 120.45823	7/21	13:59	15.3	11 FW Riffle	50.7	
W15	N - 37.66585 W - 120.45823	7/21	8:35	13.3	N - 37.66579 W - 120.45834	7/22	13:47	15.0	11 FW Riffle	50.7	7/21- recaptured a PIT tagged fish #114779, 114769, or 114734

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W16	N - 37.66585 W - 120.45823	7/21	8:35	13.3	N - 37.66579 W - 120.45834	7/22	13:47	15.0	11 FW Riffle	50.7	
W17	N - 37.66579 W - 120.45834	7/22	10:23	13.6	N - 37.66579 W - 120.45839	7/23	14:13	15.4	11 FW Riffle	50.7	
W18	N - 37.66579 W - 120.45834	7/22	10:28	13.6	N - 37.66579 W - 120.45839	7/23	14:13	15.4	11 FW Riffle	50.7	
W19	N - 37.66579 W - 120.45834	7/23	10:10	13.5	N - 37.66574 W - 120.45786	7/24	14:29	15.3	11 FW Riffle	50.7	
W20	N - 37.66579 W - 120.45834	7/23	10:27	13.5	N - 37.66574 W - 120.45786	7/24	14:29	15.3	11 FW Riffle	50.7	
W21	N - 37.66579 W - 120.45828	7/24	9:00	13.5	N - 37.66571 W - 120.45794	7/25	14:00	15.4	11 FW Riffle	50.7	7/24- recaptured PIT tag #114752
W22	N - 37.66579 W - 120.45828	7/24	9:00	13.5	N - 37.66571 W - 120.45794	7/25	14:00	15.4	11 FW Riffle	50.7	
W23	N - 37.66582 W - 120.45830	7/25	9:05	13.5	N - 37.66582 W - 120.45830	7/26	13:33	15.1	11 FW Riffle	50.7	
W24	N - 37.66582 W - 120.45830	7/25	9:05	13.6	N - 37.66582 W - 120.45830	7/26	13:33	15.1	11 FW Riffle	50.7	
W25	N - 37.66565 W - 120.45826	7/27	8:15	13.6	N - 37.66565 W - 120.45826	7/28	14:15	14.5	11 FW Riffle	50.7	
W26	N - 37.66565 W - 120.45826	7/27	8:15	13.6	N - 37.66565 W - 120.45826	7/28	14:15	14.5	11 FW Riffle	50.7	
W27	N - 37.66565 W - 120.45826	7/28	9:15	13	N - 37.66565 W - 120.45826	7/29	14:15	14.9	11 FW Riffle	50.7	
W28	N - 37.66565 W - 120.45826	7/28	9:15	13	N - 37.66565 W - 120.45826	7/29	14:15	14.9	11 FW Riffle	50.7	
W29	N - 37.66565 W - 120.45826	7/29	9:30	13.3	N - 37.66574 W - 120.45788	7/30	16:30	14.7	11 FW Riffle	50.7	7/29- recaptured PIT tag #114809
W30	N - 37.66565 W - 120.45826	7/29	9:18	13.3	N - 37.66574 W - 120.45788	7/30	16:30	14.7	11 FW Riffle	50.7	
W31	N - 37.66565 W - 120.45826	7/30	9:00	13.3	N - 37.66565 W - 120.45826	7/31	13:38	15.1	11 FW Riffle	50.7	7/30- recaptured PIT tag #114734
W32	N - 37.66565 W - 120.45826	7/30	9:07	13.3	N - 37.66565 W - 120.45826	7/31	13:38	15.1	11 FW Riffle	50.7	

Fish ID	Capture				Release				Habitat Unit ID (Stillwater 2010)	Est. RM	Comments
	Coordinates	Date	Time	Temp (°C)	Coordinates	Date	Time	Temp (°C)			
W33	N - 37.66565 W - 120.45826	7/31	9:05	13.1	N - 37.66565 W - 120.45826	8/1	13:42	15.0	11 FW Riffle	50.7	
W34	N - 37.66565 W - 120.45826	7/31	9:05	13.1	N - 37.66565 W - 120.45826	8/1	13:42	15.0	11 FW Riffle	50.7	
W35	N - 37.66565 W - 120.45826	8/1	9:02	13.2	N - 37.66565 W - 120.45826	8/2	15:40	15.8	11 FW Riffle	50.7	
W36	N - 37.66565 W - 120.45826	8/1	9:30	13.2	N - 37.66565 W - 120.45826	8/2	15:40	15.8	11 FW Riffle	50.7	
W37	N - 37.66565 W - 120.45826	8/6	9:18	13.4	--	--	--	--	11 FW Riffle	50.7	Mortality- due to chloride residue in tunnel
W38	N - 37.66565 W - 120.45826	8/6	9:28	13.4	--	--	--	--	11 FW Riffle	50.7	Mortality- due to chloride residue in tunnel
W39	N - 37.66565 W - 120.45826	8/7	9:08	13.5	N - 37.66668 W - 120.46420	8/8	17:31	15.8	11 FW Riffle	50.7	
W40	N - 37.66565 W - 120.45826	8/7	9:30	13.5	N - 37.66668 W - 120.46420	8/8	17:31	15.8	11 FW Riffle	50.7	
W41	N - 37.66643 W - 120.46432	8/8	11:18	15.5	N - 37.66643 W - 120.46432	8/9	16:00	16.7	14 BC Riffle	50.4	
W42	N - 37.66643 W - 120.46432	8/8	11:35	14.6	N - 37.66643 W - 120.46432	8/9	16:00	16.7	14 BC Riffle	50.4	
W43	N - 37.66426 W - 120.48132	8/9	11:40	17.1	N - 37.66308 W - 120.48160	8/10	15:13	18.0	25 BC Riffle	49.1	Mortality- post- swim test transport
W44	N - 37.66426 W - 120.48132	8/9	11:40	17.1	N - 37.66308 W - 120.48160	8/10	15:13	18.0	25 BC Riffle	49.1	
W45	N - 37.66565 W - 120.45826	8/13	10:25	14.4	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish not PIT tagged to limit handling after study termination per NMFS Section 10 permit conditions
W46	N - 37.66565 W - 120.45826	8/13	10:59	13.9	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish not PIT tagged to limit handling after study termination per NMFS Section 10 permit conditions
W47	N - 37.66565 W - 120.45826	8/14	9:08	13.6	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish released w/o testing per NMFS Section 10 permit conditions
W48	N - 37.66565 W - 120.45826	8/14	9:15	13.6	N - 37.66565 W - 120.45826	8/14	14:10	15.2	11 FW Riffle	50.7	Fish released w/o testing per NMFS Section 10 permit conditions

Appendix 3. PIT code and recapture table. Only five out of seven recapture fish are included in this table because PIT IDs were not recorded for two of the recaptured fish. See Figure 3 for details on the two unidentified recaptured fish, and recapture location for all recaptured fish. Days post-release is the number of days after release the PIT was recaptured.

Fish ID	PIT	Test Temp (˚C)	PIT recap freq	Days post release
W01	114756	13		
W02	114745	13		
W03	114743	13		
W04	114720	13		
W05	114764	15		
W06	--	15		
W07	114755	19		
W08	114807	19		
W09	114779	21		
W10	114773	21		
W11	114769	23		
W12	114734	23	1	11
W13	114750	17		
W14	114759	17		
W15	114741	14		
W16	114766	14		
W17	114752	16	1	1
W18	114808	16		
W19	114803	20		
W20	114723	20		
W21	114786	22		
W22	114730	22		
W23	114809	18	1	3
W24	114714	18		
W25	114787	23		
W26	114725	23		
W27	526260	17		
W28	526292	17		
W29	526299	24		
W30	526275	24		
W31	526297	19		
W32	526212	19		
W33	526226	13		
W34	526211	13		
W35	526285	25		
W36	526263	25		
W37	--	--		
W38	--	--		
W39	526255	23	1	5
W40	526298	23	1	5
W41	526227	24		
W42	526235	24		
W43	526284	25		
W44	526252	25		
W45	--	19		
W46	--	19		
W47	--	--		
W48	--	--		

Appendix 4. Experimental data table. RMR: routine metabolic rate; MMR: maximum metabolic rate; AAS: absolute aerobic scope; FAS: factorial aerobic scope; K: condition factor (mass x 10^5 / FL^3).

Fish ID	Test Temp (°C)	RMR (mg O_2 $kg^{-0.95}min^{-1}$)	MMR (mg O_2 $kg^{-0.95}min^{-1}$)	AAS (mg O_2 $kg^{-0.95}min^{-1}$)	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W01	13	1.97	7.46	5.49	3.78	112	15.7	1.12	21	9	
W02	13	2.25				110	13.3	1.00	19	9	DISCARD; tunnel leak confirmed
W03	13	1.85	6.40	4.55	3.46	102	10.9	1.03	19.5	11	
W04	13	1.75	7.12	5.37	4.08	102	10.6	1.00	19.5	14	
W05	15	2.80	7.05	4.24	2.51	113	13.4	0.93	22.0	11	
W06	15	2.37	5.98	3.61	2.52	~160	~29.2	0.87			
W07	19		9.79			126	21.4	1.05	22.5	12	DISCARD; activity during RMR
W08	19		6.41			100	10.5	1.07	18.0	9	DISCARD; activity during RMR
W09	21	3.96	11.19	7.23	2.82	125	20.2	1.03	24.0	12	
W10	21	2.86	8.66	5.80	3.03	197	79.6	1.04	36.0	20	
W11	23	3.94	10.99	7.05	2.79	132	24.3	1.06	21.0	12	
W12	23	3.88	8.73	4.85	2.25	131	25.1	1.12	24.0	13	
W13	17	1.89				141	29.4	1.05	26.0	14	DISCARD; no MR increase with velocity 33 to 53 cms^{-1}
W14	17	2.47				142	29.9	1.04	23.0	10	DISCARD; no MR increase with velocity 30 to 46 cms^{-1}
W15	14	2.14				129	22.2	1.03	26.0	11	DISCARD; no MR increase with velocity 32 to 46 cms^{-1}
W16	14	2.53	5.61	3.08	2.22	137	28.4	1.10	24.0	12	
W17	16		8.13			135	27.6	1.12	26.0	13	DISCARD; activity during RMR
W18	16	2.31	8.26	5.95	3.58	133	25.9	1.10	25.0	10	
W19	20	3.75	9.95	6.19	2.65	147	38.4	1.21	28.0	11	
W20	20	3.66	10.83	7.16	2.96	134	28.1	1.17	25.0	11	
W21	22	3.09	11.15	8.06	3.61	124	21.7	1.14	21.0	10	

Fish ID	Test Temp (°C)	RMR (mg O_2 $kg^{-0.95}min^{-1}$)	MMR (mg O_2 $kg^{-0.95}min^{-1}$)	AAS (mg O_2 $kg^{-0.95}min^{-1}$)	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W22	22	2.89	9.73	6.84	3.37	115	15.8	1.04	19.0	8	
W23	18	2.73	9.35	6.62	3.42	164	47.1	1.07	30.0	18	
W24	18	2.81	6.97	4.16	2.48	133	22.6	0.96	21.0	13	
W25	23	4.11	11.23	7.12	2.73	121	18.7	1.06	20.0	11	
W26	23	3.90	9.43	5.53	2.42	129	23.4	1.09	23.0	12	
W27	17	2.76	9.57	6.81	3.47	134	24.9	1.03	21.0	13	
W28	17	2.87	8.69	5.81	3.02	122	19.9	1.10	24.0	12	
W29	24	5.31	13.41	8.10	2.52	104	13.0	1.16	18.0	10	
W30	24	5.26	9.17	3.91	1.74	115	16.5	1.08	19.0	12	
W31	19	2.81	8.07	5.26	2.87	138	29.0	1.10	24.0	10	
W32	19	3.21	6.71	3.51	2.09	140	27.2	0.99	28.0	11	
W33	13	2.17	6.97	4.80	3.21	117	16.4	1.02	19.0	8	
W34	13	2.02	6.40	4.38	3.17	105	12.2	1.05	19.0	7	
W35	25	4.87	10.09	5.21	2.07	130	27.4	1.25	26.0	10	
W36	25	7.01	13.12	6.11	1.87	111	12.4	0.91	17.0	7	
W37	Mortality- due to chloride residue in tunnel										
W38	Mortality- due to chloride residue in tunnel										
W39	23	3.76	7.11	3.36	1.89	101	12	1.02	17.0	6	
W40	23	4.76	14.41	9.65	3.03	122	18.5	1.16	20.0	10	
W41	24	4.87	10.04	5.17	2.06	131	23.1	1.03	22.0	12	
W42	24	3.94	10.04	6.10	2.55	138	25.5	0.97	22.0	12	
W43	25	5.54	9.03	3.49	1.63	107	14.5	1.18	19.0	8	
W44	25	6.13	12.61	6.48	2.06	113	14.9	1.03	19.0	8	
W45	19	3.49	11.76	8.27	3.37	~101	~11.5	1.12	~16	~10	
W46	19	3.51	7.59	4.08	2.16	~108	~13.1	1.04	~17	~10	

Fish ID	Test Temp (°C)	RMR (mg O_2 $kg^{-0.95}min^{-1}$)	MMR (mg O_2 $kg^{-0.95}min^{-1}$)	AAS (mg O_2 $kg^{-0.95}min^{-1}$)	FAS	FL (mm)	Mass (g)	K	Body Depth (mm)	Body Width (mm)	Quality Control
W47	Fish released w/o testing per NMFS Section 10 permit conditions										
W48	Fish released w/o testing per NMFS Section 10 permit conditions										

From: Staples, Rose
BCC To: Don Pedro Relicensing Participants Email Group
Sent: Friday, February 27, 2015 2:12 PM
Cc: Staples, Rose <Rose.Staples@hdrinc.com>
Subject: LTR IFM-Evaluation of Effective Usable Habitat Area for Over-Summering O.mykiss Report for 30-Day Review

Please find attached for your review and comment the *Lower Tuolumne River Instream Flow Study-Evaluation of Effective Usable Habitat Area for Over-summering O.mykiss* report. This report is the final component of the multi-subject IFIM required by FERC's July 2009 Order. The history of all the study components is provided in the background section of this report. Please provide any comments you might have to me by March 27, 2015. Thank you.

Rose Staples, CAP-OM
Executive Assistant

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DRAFT REPORT ◦ FEBRUARY 2015

Lower Tuolumne River Instream Flow Study—Evaluation of Effective Usable Habitat Area for over-summering *O. mykiss*

PREPARED FOR

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Suggested citation:
Stillwater Sciences. 2015. Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering *O. mykiss*. Draft Report. Prepared by Stillwater Sciences, Davis, California for Turlock Irrigation District, Turlock California and Modesto Irrigation District, Modesto, California.

Cover photo:
Habitat suitability criteria site-specific survey on the lower Tuolumne River, May 2012.

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1 BACKGROUND

Per Federal Energy Regulatory Commission (Commission) Order issued on July 16, 2009, the Turlock Irrigation District and Modesto Irrigation District (collectively: Districts) in consultation with resource agencies, were required "to develop and implement an instream flow incremental methodology (IFIM) study to determine instream flows necessary to maximize Chinook salmon (*Oncorhynchus tshawytscha*) and Central Valley steelhead (*O. mykiss*) production and survival throughout various life stages. The results of the physical habitat simulation (PHABSIM) flow model under the IFIM framework would assist in identifying the amount of available habitat (weighted usable area) for the species under various flow conditions." In addition, the Order required the Districts to develop a water temperature model in conjunction with the instream flow study "to determine the downstream extent of thermally suitable habitat to protect summer juvenile *Oncorhynchus mykiss* rearing under various flow conditions and to determine flows necessary to maintain water temperatures at or below 68 degrees Fahrenheit from La Grange Dam to Roberts Ferry Bridge."

On October 14, 2009, the Districts submitted to the Commission two study plans; the *Lower Tuolumne River Instream Flow Studies – Final Study Plan* ("IFIM Study Plan") (Stillwater Sciences 2009a) and the *Lower Tuolumne River Water Temperature Modeling – Final Study Plan* (Water Temperature Model Study Plan) (Stillwater Sciences 2009b). The IFIM Study Plan and the Water Temperature Model Study were modified and approved, pursuant to the Commission's May 12, 2010 Order.

In order to examine the broad flow ranges identified in the Commission's July 16, 2009 Order, the IFIM Study Plan separated the study into two separate investigations: (1) A conventional one-dimensional (1-D) PHABSIM model which examined in-channel habitat conditions affecting Chinook salmon (*O. tshawytscha*) and Central Valley steelhead (*O. mykiss*) at flows from approximately 100–1,000 cfs and (2) a 2-D hydraulic model of overbank areas, as well as adjacent in-channel locations, for flows of 1,000–5,000 cfs. The *Lower Tuolumne River Instream Flow Studies – Pulse Flow Study Report* (Stillwater Sciences 2012) covering the 2-D hydraulic model of overbank areas was filed with the Commission on June 18, 2012. The *Lower Tuolumne River Instream Flow Study – Final Report* (Stillwater Sciences 2013a) covering 1-D PHABSIM modeling of in-channel conditions was filed with the Commission on April 26, 2013.

The Water Temperature Model Study Plan approved by the May 12, 2010 Commission Order was satisfied with the *Tuolumne River Water Temperature Modeling Study–Final Report* submitted on March 11, 2011 (Stillwater Sciences 2011). The 2011 report incorporated the HEC-5Q water temperature model that was developed for the Tuolumne River and other tributaries of the San Joaquin River with CALFED funding (RMA 2008). Subsequent to the filing of the 2011 water temperature study report, the Lower Tuolumne River Temperature Model (TID/MID 2013) was developed during the Don Pedro Hydroelectric Project relicensing process. The 2013 model was developed specifically for the lower Tuolumne River using the HEC-RAS platform and features improved calibration performance and connectivity to reservoir operations not found in the HEC-5Q model.

As described in the IFIM Study Plan, this report fulfils the remaining requirements of the Commission's May 12, 2010 Order pertaining to the instream flow study and presents a summertime water temperature suitability component for fry, juvenile, and adult *O. mykiss* that integrates both hydraulic and thermal habitat considerations. The results from the Lower Tuolumne River Temperature Model (TID/MID 2013) over a range of flows were combined with results from *Instream Flow Study* 1-D PHABSIM model results (Stillwater Sciences 2013a) to examine the downstream extent of thermally suitable habitat. The Lower Tuolumne River Temperature Model (TID/MID 2013) was used to assess flow and air temperature conditions necessary to maintain various water temperature thresholds (including 20°C [68°F]) at varying downstream locations, including Robert's Ferry Bridge (RM 39.5), as required by the Commission's July 2009 Order.

2 OBJECTIVES

The objective of this evaluation is to estimate the "effective" weighted usable area (eWUA) of select lower Tuolumne River habitat reaches for various life history stages of *O. mykiss* during the summer months (i.e., June-September). The evaluation of eWUA is an alternate depiction of the traditional weighted usable area (WUA) vs. flow relationship used in stream habitat analysis, which is traditionally based upon physical (i.e., depth, velocity, and/or substrate and cover) parameters (Bovee 1982). Depending on thermal conditions during summertime, the total usable area in a river reach for rearing *O. mykiss* (WUA multiplied by the length of the reach) at a given flow may be lower than depicted by the standard WUA vs. flow relationship if temperatures are unsuitable. The combined influences of hydraulic habitat suitability and thermal suitability for a given *O. mykiss* life stage (i.e., fry, juvenile, and adult) is quantified and described in this report as eWUA.

Flow in the lower Tuolumne River necessary to maintain specified downstream water temperatures can be greatly influenced by diurnal maximum air temperatures, especially during summer months (June–September). The current Lower Tuolumne River Temperature Model (TID/MID 2013) is used to provide supplemental information on the effects of maximum air temperatures on modeled water temperatures and to provide the thermal conditions for use in analyzing eWUA.

3 METHODS

3.1 Temperature Evaluation Thresholds

The primary metric used in this analysis to assess thermal suitability for over-summering *O. mykiss* is the maximum weekly average temperature (MWAT). The MWAT is a commonly used measure of chronic (i.e., sub-lethal) exposure when considering the effect of temperature on salmonids (Carter 2005). In this analysis, a MWAT threshold of 20°C (68°F) was evaluated, as directed in the July 16, 2009 Order. Although the majority of historical (1996–2009) snorkel survey observations of *O. mykiss* in the lower Tuolumne River have occurred at temperatures of 20°C (68°F) or below (Ford and Kirihara 2010), *O. mykiss* have been routinely observed occupying Tuolumne River habitats at temperatures ranging from 11–25°C (52–77°C). Using wild juvenile *O. mykiss* collected from the Tuolumne River in the summer of 2014, a recently completed thermal performance study (Farrell et al. 2014) found a peak in the absolute aerobic scope[1] (AAS) vs. temperature curve at 21.2°C (70°F), higher than the 19°C (66°F) growth rate

[1] Aerobic scope is defined here as the difference between resting and maximal oxygen consumption rates of swimming fish at various temperatures and relies upon an assumption that biochemical and physiological capacities of salmonids have evolved to optimize fitness related performance (e.g., growth, locomotion) within a particular temperature range.

optimum identified by Myrick and Cech (2001). Because Farrell et al. (2014) also found that the AAS of the wild *O. mykiss* test fish remained within 5% of the peak AAS between 17.8°C (64°F) to 24.6°C (76°F), these site-specific empirical data with broader temperature thresholds were selected for evaluation of thermal suitability for *O. mykiss*. In the current study, the temperatures of 18°C (66.4°F), 20°C (68°F), 22°C (71.6°F), and 24°C (75.2°F) were evaluated over each of the summer months (June through September) when these temperatures can be exceeded in the lower Tuolumne River.

3.2 Physical Habitat Modeling

The WUA results for this analysis were based on the PHABSIM model as described in the *Lower Tuolumne River Instream Flow Study–Final Report* (Stillwater Sciences 2013a). The results from this model provide estimates of physical habitat for *O. mykiss* life stages over a range of constant flow simulations from 50–1,200 cfs, incorporating eight macrohabitat types and utilizing consensus-based habitat suitability criteria validated by site-specific field observations. The overall study reach for the analyses in the report extended from RM 51.9 downstream to near the city of Waterford, CA (RM 29). Finer sub-reach divisions were developed for the current study to allow for more detailed analysis of the usable habitat areas and related temperature conditions on a sub-reach basis. Using the PHABSIM model sub-reach divisions shown in Table **3-1**, Appendix A provides estimates of WUA (ft^2/1,000 ft) for each life history stage for each sub-reach over a discharge range of 50–1,200 cfs.

Table 3-1. Lower Tuolumne River PHABSIM sub-reach model boundaries.

Sub-reach model	Upstream RM	Downstream RM	Distance (feet)
1 (La Grange powerhouse to Basso Bridge)	51.9	46.9	26,400
2 (Basso Bridge. to Bobcat Flat)	46.9	43.1	46,464
3 (Bobcat Flat to Roberts Ferry Bridge)	43.1	39.5	64,944
4 (Roberts Ferry Bridge to Waterford)	39.5	29.1	120,384

Transect weighting within each sub-reach model reflects the percent occurrence (by length) of macrohabitats found within that sub-reach (Appendix B). To allow more precise sub-reach estimates to be combined with the spatially explicit HEC-RAS temperature model (HDR 2013) results, summation of the sub-reach-specific WUA (ft^2/1,000 ft) and channel length (ft) product across 0.1 mile increments from RM 51.9 to RM 29.1 was used to estimate the total amount of usable habitat for each life stage within the study reach.

3.3 Water Temperature Model

The HEC-RAS version of the Lower Tuolumne River Temperature Model (TID/MID 2013) was used to provide daily water temperature predictions at 0.5 mile increments from RM 51.9 downstream to near Waterford, CA (RM 29) under steady flow releases ranging from 100–1,200 cfs. These model runs incorporated historical meteorology data over a 42-year period of record dating from October 1970 through September 2012. Modeling results were also used to develop relationships between water temperature, air temperature and discharge at the downstream ends of the four sub-reach boundaries (RM 46.9, RM 43.1, RM 39.5, and RM 29.1).

3.4 Effective Weighted Useable Area Analysis

For each modeled constant flow release (100–1,200 cfs), the HEC-RAS water temperature model results (Section 3.3) were accumulated over the 42 year period of record at 0.5 mile intervals. Using linear interpolation, MWATs were then determined for each summer month at 0.1 RM intervals along with how often the MWAT thresholds were exceeded for each location and month within the period of record. To represent average conditions, the location at which the MWAT threshold was exceeded in half (21 of the 42) of the annual results was used in subsequent eWUA estimates. Calculations of eWUA were made using PHABSIM modeling results over the same 0.1 RM intervals, excluding sub-reach segments where the MWAT threshold was not met in 50% of the years simulated. Four distinct sub-reach combinations were developed to reflect cumulative eWUA at various RM locations in the lower Tuolumne River PHABSIM study area. These combinations included; Sub-reach 1 (RM 51.9 to RM 46.9), Sub-reach 1–2 (RM 51.9 to RM 43.1), Sub-reach 1–3 (RM 51.9 to RM 39.5), and Sub-reach 1–4 (RM 51.9 to RM 29.1).

4 RESULTS

The water temperature modeling results were combined with PHABSIM modeling results to allow more precise estimates of (1) the length of river channel meeting a 68°F (20°C) MWAT (maximum weekly average temperature) threshold as well as lower (18°C [66.4°F]) and higher thresholds (22°C [71.6°F] or 24°C [75.2°F]); (2) relationships between air temperature and river temperature; and (3) the combined temperature/habitat (eWUA) results for juvenile and adult life stages of oversummering *O. mykiss*.

4.1 Water Temperature Model Results

Daily maximum air temperature during summer months (June-September) over a 42-year period of record dating from October 1970 through September 2012 are shown in Figure 4-1. These results show that July has the greatest number of days where air temperatures exceed 35°C (95°F). The effect of daily maximum air temperatures on predicted daily average water temperatures over a range of flows at various RM locations associated with the downstream boundary of each sub-reach is shown in Figures 4-2 through 4-5. For example, using a daily average water temperature objective of 20°C (68°F) at Robert's Ferry Bridge (RM 39.5) and assuming a maximum daily air temperature of 35°C (95°F), Figure 4-4 shows that this water temperature threshold would be met at a flow release of approximately 300 cfs. However, higher water temperature objectives of 22°C (71.6°F) or 24°C (75.2°F) could be met at RM 39.5 with a flow release of 200 cfs or 150 cfs, respectively. The river flow necessary to attain these same temperature objectives farther downstream at RM 29 would be 600 cfs and 425 cfs, respectively, approximately 300 percent greater.

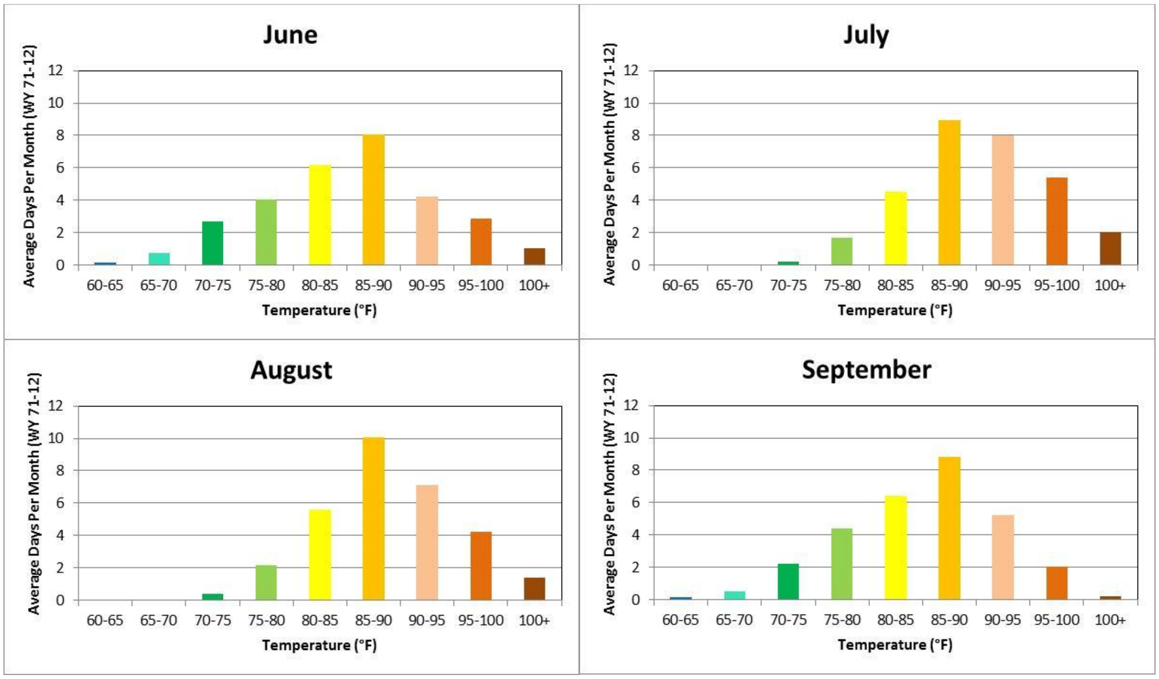

Figure 4-1. Daily maximum air temperatures from the Lower Tuolumne River temperature model (1970-2012).

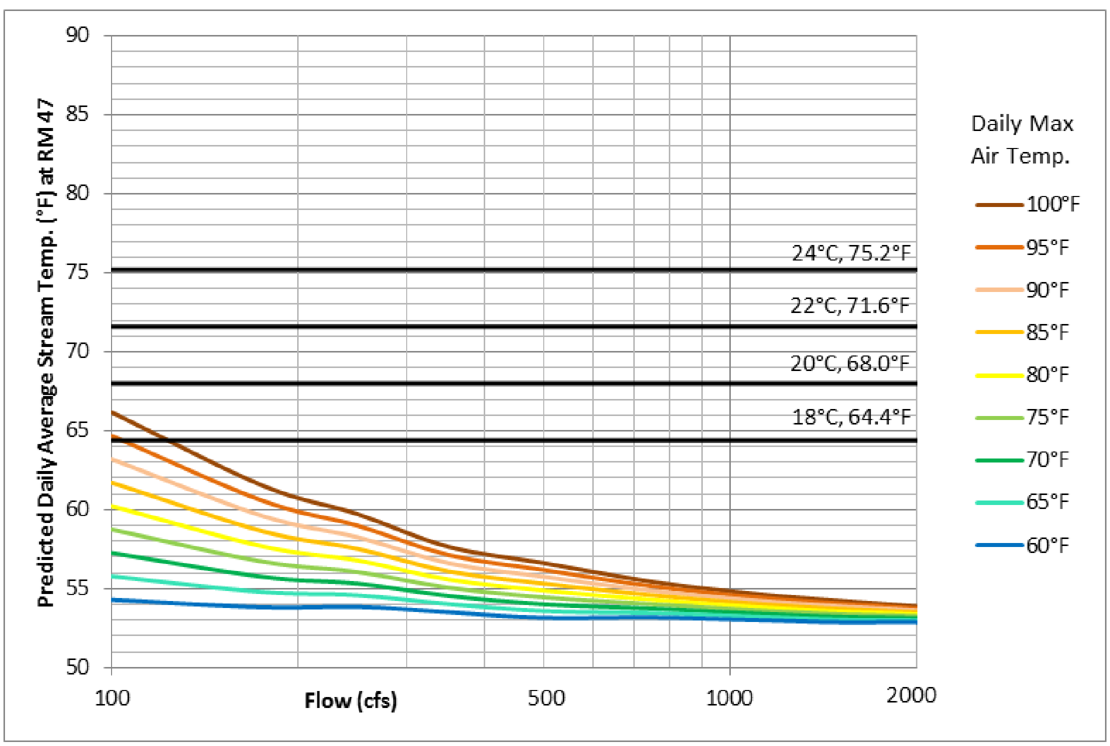

Figure 4-2. Modeled Tuolumne River daily average water temperature associated with daily maximum air temperature over a range of flows at RM 47.

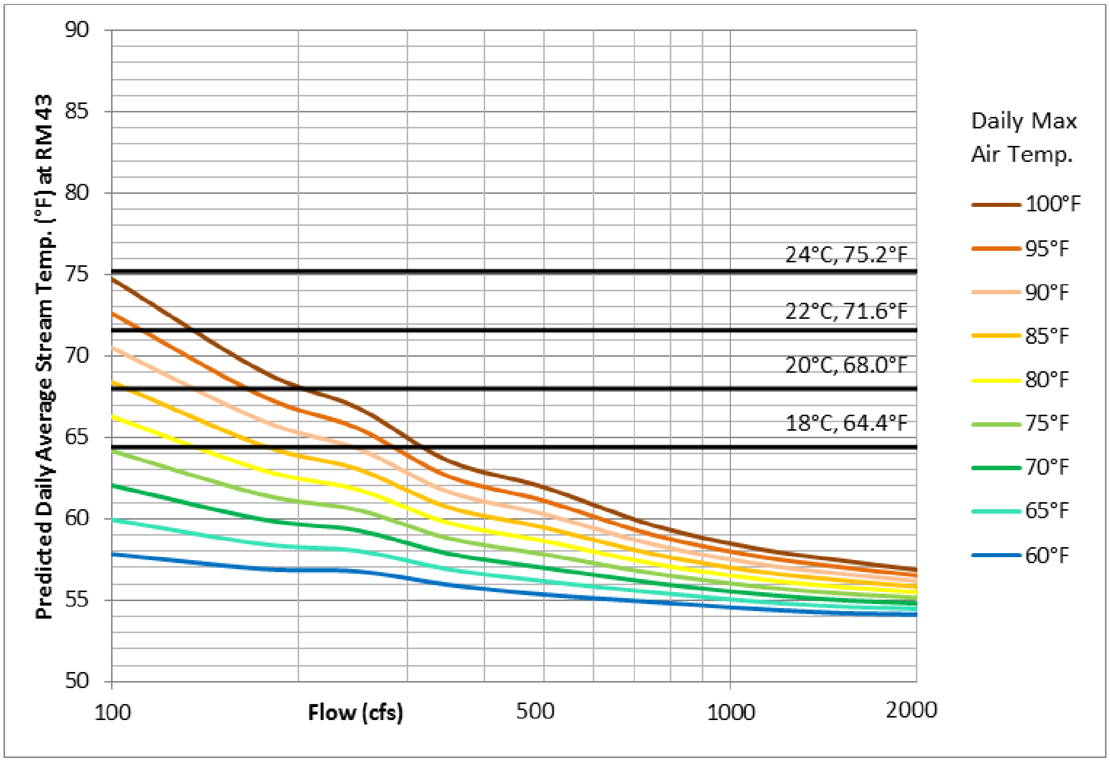

Figure 4-3. Modeled Tuolumne River daily average water temperature associated with daily maximum air temperature over a range of flows at RM 43.

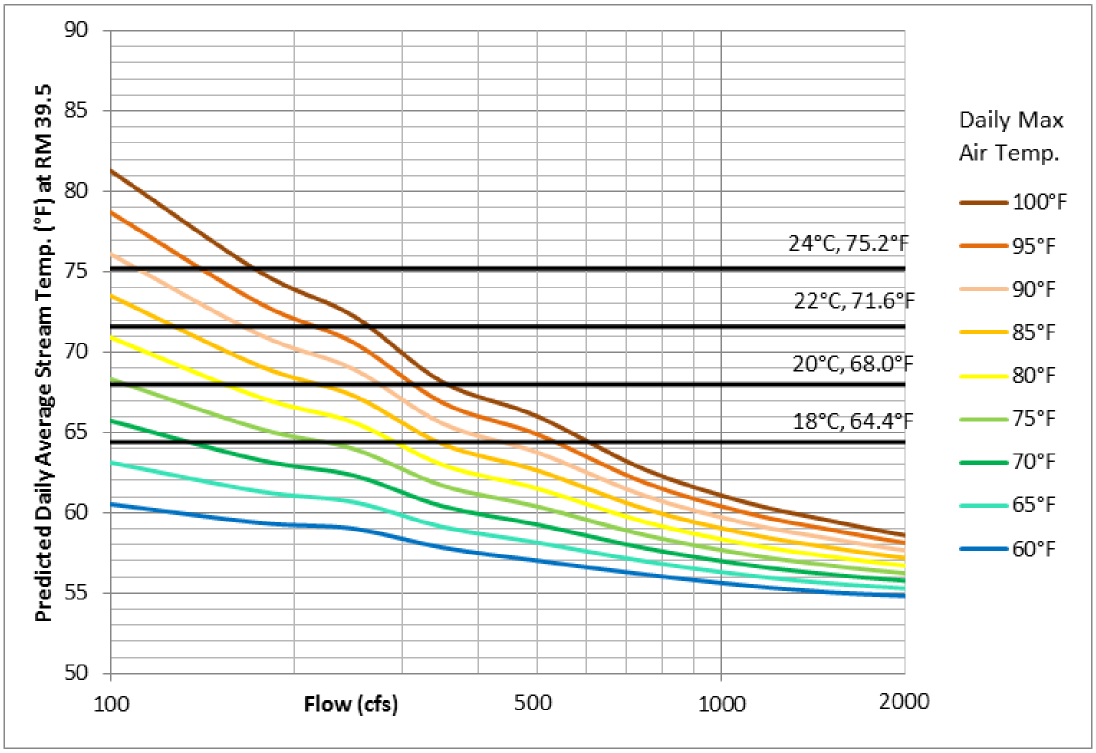

Figure 4-4. Modeled Tuolumne River daily average water temperature associated with daily maximum air temperature over a range of flows at RM 39.5.

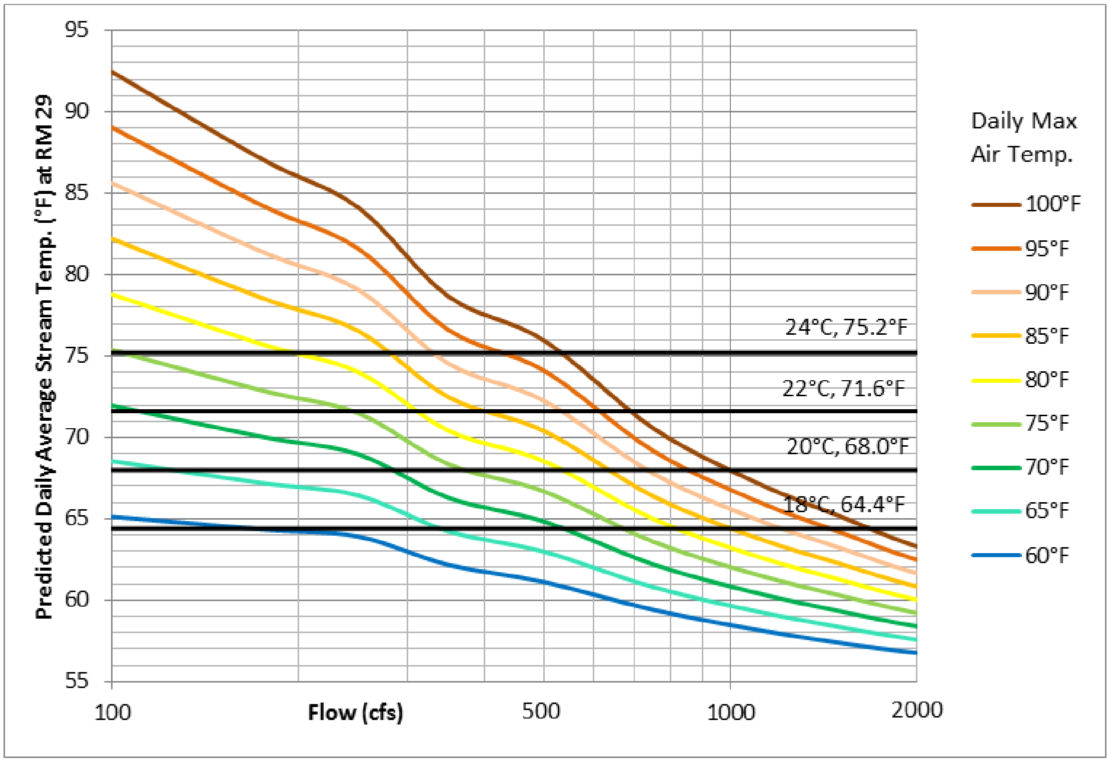

Figure 4-5. Modeled Tuolumne River daily average water temperature associated with daily maximum air temperature over a range of flows at RM 29.0

4.2 Effective Usable Habitat by River Mile

The cumulative effective usable habitat for *O. mykiss* life stages computed for various sub-reaches in the lower Tuolumne River during summer months under each of the MWAT thresholds is shown in Appendix C, Figures C-1 through C-48. The figures are compiled by life stage (fry, juvenile, adult) and reach designation for each summer month and include total usable habitat with no temperature threshold applied.

Applying each of the four MWAT temperature thresholds, the eWUA results show that for habitats downstream to RM 46.9 (Sub-reach 1), there is no change in effective usable area under any MWAT threshold over the entire range of simulated flows for all life stages in any month. (Figures C-1, C-5, C-9, C-13, C-17, C-21, C-25, C-29, C-33, C-37, C-41, and C-45).

For habitats downstream to RM 43.1 (Sub-reaches 1 and 2), there is no change in effective usable area with MWAT thresholds greater than 18°C (64.4°F) over the entire range of simulated flows for all life stages in any month. Effective usable habitat is reduced with a MWAT threshold of 18°C (64.4°F) at flows less than 150 cfs, with the largest reductions occurring in fry and juvenile habitat during July. (Figures C-2, C-6, C-10, C-14, C-18, C-22, C-26, C-30, C-34, C-38, C-42, and C-46).

For habitats downstream to RM 39.5 (Sub-reaches 1, 2, and 3), there is no change in effective usable area with MWAT thresholds greater than 22°C (71.6°F) over the entire range of simulated flows for all life stages in any month. Effective usable habitat is slightly reduced with a MWAT threshold of 20°C (68°F) at flows less than 175 cfs, with the largest reductions occurring in fry and juvenile habitat during July. Correspondingly, effective usable habitat is further reduced with a MWAT threshold of 18°C (64.4°F) at flows less than 250 cfs, again with the largest reductions occurring in fry and juvenile habitat during July (Figures C-3, C-7, C-11, C-15, C-19, C-23, C-27, C-31, C-35, C-39, C-43, and C-47).

For habitats downstream to RM 29.1 (Sub-reaches 1 through 4), there are reductions in effective usable area shown under all MWAT thresholds except during September with a MWAT threshold of 24°C (75.2°F). During the warmest (July) conditions, flows up to 200 cfs are required to maintain predicted effective usable habitat for all life stages with a MWAT threshold of 24°C (75.2°F), with associated flows up to 300 cfs with a MWAT threshold of 22°C (71.6.°F), 425 cfs with a threshold of 20°C (68°F), and 700 cfs with a MWAT threshold of 18°C (64.4°F) (Figures C-4, C-8, C-12, C-16, C-20, C-24, C-28, C-32, C-36, C-40, C-44, and C-48).

5 DISCUSSION

1-D PHABSIM physical habitat modeling at flows from 100–1,200 cfs was combined with HEC-RAS water temperature modeling over a 42-year period of record meteorology (1970–2012) to provide estimates of eWUA in the lower Tuolumne River meeting a range of MWAT thresholds ranging from 18–24°C (64.4–75.2°F) during summer (June–September). For summertime flow ranges from 100 to 250 cfs, thermally suitable habitat is maintained across all months and over all MWAT thresholds downstream to Basso Bridge (RM 46.9), with incremental reductions in usable habitat downstream to Waterford (RM 29.1) as MWAT thresholds decrease. Applying a MWAT temperature threshold of 20°C (68°F), summertime flows of 150 cfs and above would maintain thermally suitable habitat for all life stages of *O. mykiss* downstream to Roberts Ferry Bridge (RM 39.5). However, this estimate is highly sensitive to daily maximum air temperatures and can range up to approximately 350 cfs when air temperatures exceed 37.8°C (100°F).

As discussed in the *Assessment of Don Pedro Project Operations to Meet EPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards* (TID/MID 2014, Attachment A), potential re-operation of the Don Pedro Project to meet USEPA (2003) temperature recommendations was previously shown to be infeasible under a range of potential scenarios evaluated. HEC-RAS water temperature modeling showed that application of the USEPA (2003) water temperature recommendations would result in limitation of thermally suitable habitat for *O. mykiss* fry and juvenile life stages to only the first few miles downstream of La Grange Diversion Dam (RM 52.2) during summer. The Districts have recently completed a study of thermal performance of wild juvenile *O. mykiss* in the lower Tuolumne River (Farrell et al. 2014), which has provided specific empirical data to better evaluate site-specific water temperature objectives for the lower Tuolumne River. Results of Farrell et al. were used to improve the scope of the eWUA analyses completed in this document. For example, the empirical data obtained by testing wild Tuolumne River juvenile *O. mykiss* demonstrated that fish tested at 24°C (75.2°F) performed nearly as well as fish tested at cooler temperatures and attained AAS within 5% of the peak values estimated. Using this temperature as an upper threshold, thermally suitable conditions could be maintained at RM 39.5 using flows of 150 cfs even when maximum daily air temperatures exceeded 37.8°C (100°F)(see Figure 4-4).

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Appendices

Appendix A

1-D PHABSIM Weighted Usable Area Results by Sub-reach in the lower Tuolumne River

Table A-1. Weighted usable area (sq ft/1,000 ft) results for *O. mykiss* in Sub-reach 1 (RM 51.9 to RM 46.9).

Simulated discharge (cfs)	*O. mykiss* fry	*O. mykiss* juvenile	*O. mykiss* adult
50	60,590	55,485	13,876
75	55,359	59,745	18,777
100	51,170	62,297	22,995
125	48,063	63,790	26,694
150	46,013	64,422	29,806
175	44,051	64,404	32,481
200	42,554	63,738	34,721
225	41,319	63,016	36,720
250	40,134	62,088	38,455
275	38,918	61,093	39,991
300	37,850	60,075	41,240
325	36,988	59,046	42,258
350	36,195	57,943	43,101
375	35,445	56,796	43,845
400	34,878	55,625	44,464
425	34,367	54,549	44,987
450	33,974	53,621	45,429
475	33,774	52,604	45,789
500	33,509	51,735	46,067
550	33,114	50,242	46,486
600	33,134	48,734	46,734
650	33,392	47,485	46,864
700	33,323	46,291	46,872
750	33,381	45,093	46,862
800	33,567	44,222	46,856
850	33,280	43,494	46,775
900	33,393	43,073	46,657
1000	33,574	42,207	46,320
1100	33,465	41,953	45,865
1200	33,933	41,952	45,387

Table A-2. Weighted usable area (sq ft/1,000 ft) results for *O. mykiss* in Sub-reach 2 (RM 46.9 to RM 43.1).

Simulated discharge (cfs)	*O. mykiss* fry	*O. mykiss* juvenile	*O. mykiss* adult
50	50,517	49,690	15,316
75	46,057	52,039	20,459
100	42,868	53,226	24,665
125	40,569	53,877	28,173
150	39,036	54,007	30,916
175	37,600	53,636	32,962
200	36,312	52,938	34,489
225	35,326	52,331	35,736
250	34,484	51,590	36,785
275	33,575	50,824	37,686
300	32,812	50,108	38,380
325	32,252	49,306	38,885
350	31,764	48,417	39,256
375	31,328	47,545	39,584
400	31,000	46,709	39,835
425	30,711	45,923	40,037
450	30,492	45,279	40,209
475	30,427	44,571	40,337
500	30,135	43,909	40,437
550	29,626	42,848	40,587
600	29,487	41,770	40,654
650	29,743	41,048	40,665
700	29,910	40,248	40,601
750	30,558	39,435	40,509
800	31,405	38,928	40,437
850	31,699	38,559	40,300
900	32,537	38,367	40,150
1000	34,155	38,308	39,811
1100	34,792	38,559	39,446
1200	36,261	39,207	39,113

Table A-3. Weighted usable area (sq ft/1,000 ft) results for *O. mykiss* in Sub-reach 3 (RM 43.1 to RM 39.5).

Simulated discharge (cfs)	*O. mykiss* fry	*O. mykiss* juvenile	*O. mykiss* adult
50	53,089	51,063	15,649
75	49,432	53,708	21,136
100	46,056	55,127	25,671
125	43,289	56,108	29,431
150	41,124	56,469	32,330
175	39,009	56,283	34,529
200	37,073	55,771	36,156
225	35,568	55,267	37,433
250	34,319	54,518	38,468
275	33,070	53,730	39,290
300	32,087	52,963	39,906
325	31,400	52,056	40,311
350	30,842	51,013	40,562
375	30,352	49,980	40,733
400	29,980	49,028	40,790
425	29,660	48,094	40,789
450	29,412	47,281	40,734
475	29,342	46,398	40,631
500	29,045	45,554	40,518
550	28,510	44,103	40,210
600	28,399	42,626	39,827
650	28,647	41,606	39,414
700	28,787	40,519	38,991
750	29,387	39,405	38,595
800	30,191	38,650	38,277
850	30,539	38,063	37,930
900	31,428	37,613	37,624
1000	33,138	37,174	37,019
1100	33,886	37,106	36,481
1200	35,450	37,424	35,995

Table A-4. Weighted usable area (sq ft/1,000 ft) results for *O. mykiss* in Sub-reach 4 (RM 39.5 to RM 29.1).

Simulated discharge (cfs)	*O. mykiss* fry	*O. mykiss* juvenile	*O. mykiss* adult
50	53,629	53,735	15,807
75	49,676	56,226	21,159
100	46,284	57,463	25,663
125	43,822	58,259	29,443
150	41,980	58,526	32,417
175	40,229	58,281	34,766
200	38,639	57,615	36,649
225	37,432	57,034	38,324
250	36,438	56,290	39,779
275	35,409	55,562	41,065
300	34,536	54,826	42,090
325	33,870	54,009	42,893
350	33,296	53,076	43,546
375	32,767	52,119	44,123
400	32,348	51,231	44,598
425	31,991	50,400	45,012
450	31,725	49,675	45,359
475	31,614	48,904	45,635
500	31,289	48,172	45,859
550	30,709	46,962	46,208
600	30,488	45,724	46,448
650	30,488	44,787	46,612
700	30,417	43,857	46,687
750	30,709	42,957	46,725
800	31,117	42,288	46,767
850	31,256	41,763	46,747
900	31,814	41,354	46,689
1000	32,957	40,652	46,438
1100	33,314	40,453	46,104
1200	34,176	40,607	45,694

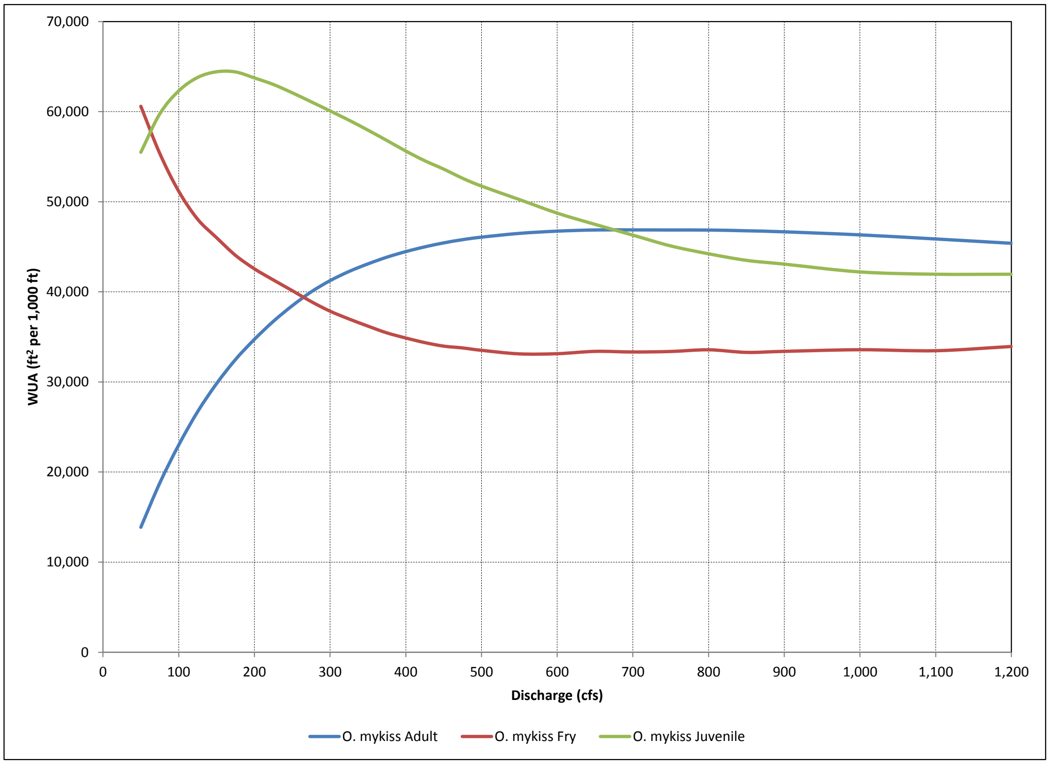

Figure A-1. *O. mykiss* weighted usable area in Sub-reach 1 (RM 51.9 to RM 46.9).

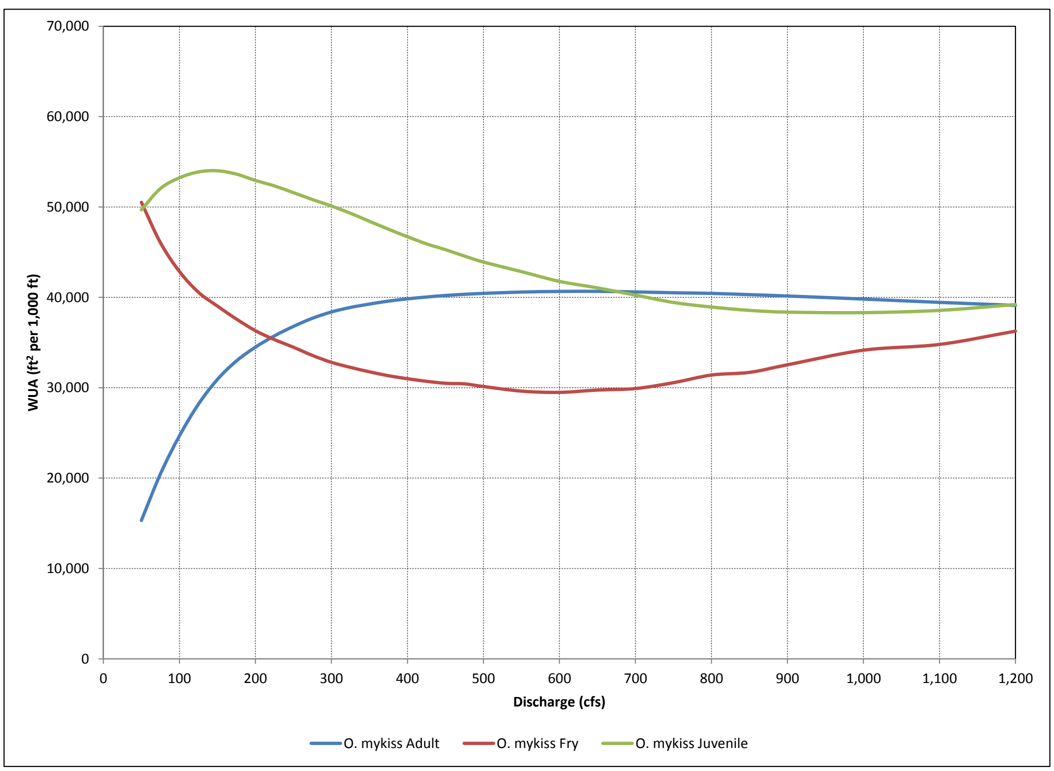

Figure A-2. *O. mykiss* weighted usable area in Sub-reach 2 (RM 46.9 to RM 43.1).

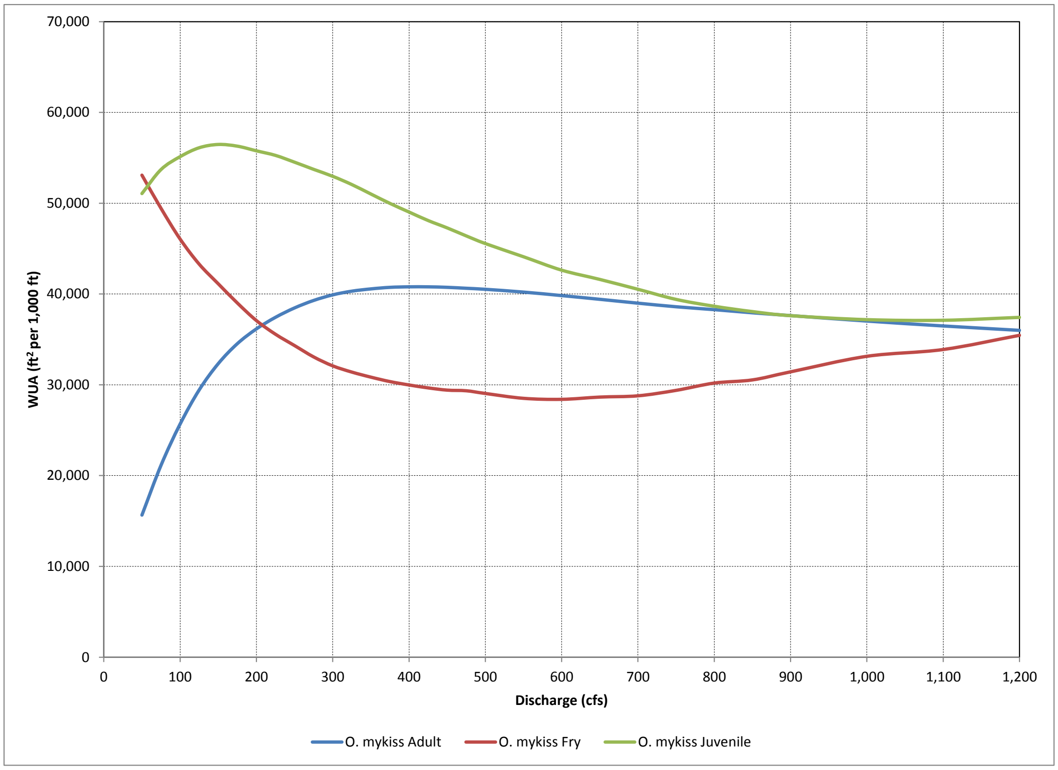

Figure A-3. *O. mykiss* weighted usable area in Sub-reach 3 (RM 43.1 to RM 39.5).

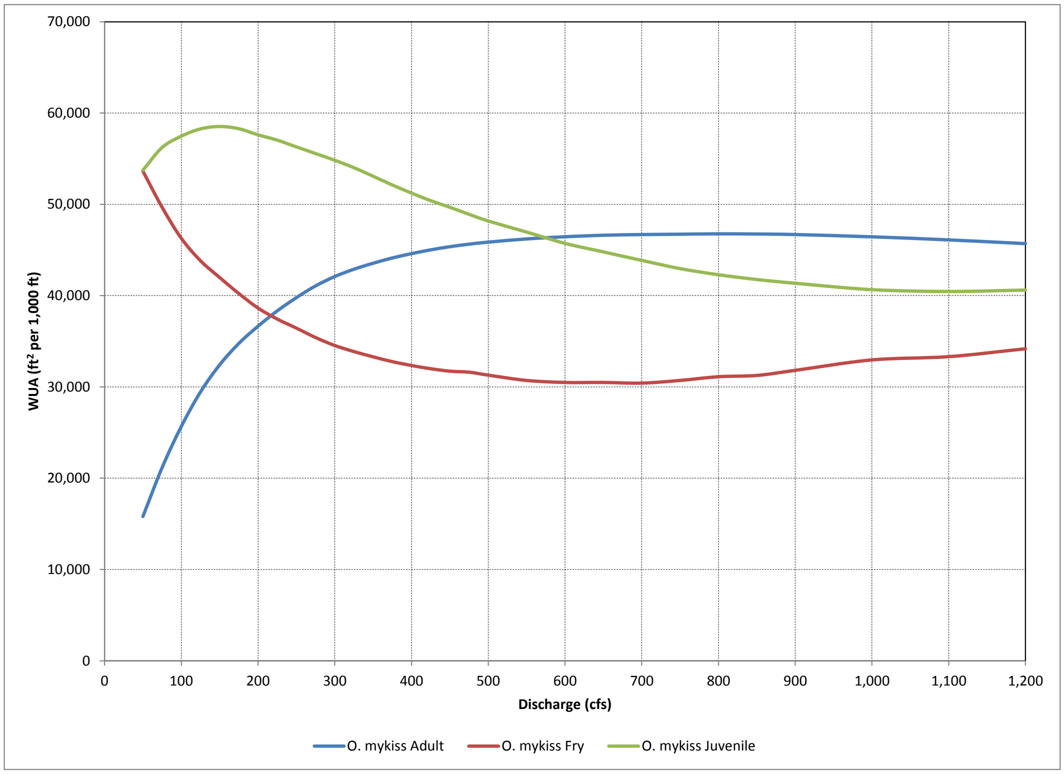

Figure A-4. *O. mykiss* weighted usable area in Sub-reach 4 (RM 39.5 to RM 21.9).

Appendix B

1-D PHABSIM Transect Weighting by Sub-reach in the lower Tuolumne River

Table B-1 Transect weighting used for reach models 1–4 (RM 52.2–24.6).

Sub-reach	Channel form	Habitat type	Length (ft)	% of total	Transects	Weight per transect	Weight per habitat
1	Bar Complex	Glide	226	0.88%	2	0.44%	0.88%
		Pool	739	2.87%	5	0.57%	2.87%
		Riffle	3,050	11.87%	7	1.70%	11.87%
		Run	2,027	7.89%	6	1.31%	7.89%
	Flatwater	Glide	1,758	6.84%	3	2.28%	6.84%
		Pool	6,714	26.12%	6	4.35%	26.12%
		Riffle	4,532	17.63%	2	8.81%	17.63%
		Run	6,659	25.91%	9	2.88%	25.91%
Sub-reach 1 (RM 51.9–46.9) Total			**25,705**	**100.00%**	**40**		**100.00%**
2	Bar Complex	Glide	0	0.00%	2	0.00%	0.00%
		Pool	561	2.62%	5	0.52%	2.62%
		Riffle	4,303	20.07%	7	2.87%	20.07%
		Run	4,927	22.98%	6	3.83%	22.98%
	Flatwater	Glide	126	0.59%	3	0.20%	0.59%
		Pool	2,886	13.46%	6	2.24%	13.46%
		Riffle	575	2.68%	2	1.34%	2.68%
		Run	8,059	37.59%	9	4.18%	37.59%
Sub-reach 2 (RM 46.9–43.1) Total			**21,437**	**100.00%**	**40**		**100.00%**
3	Bar Complex	Glide	572	3.31%	2	1.66%	3.31%
		Pool	1,410	8.16%	5	1.63%	8.16%
		Riffle	3,394	19.65%	7	2.81%	19.65%
		Run	4,281	24.78%	6	4.13%	24.78%
	Flatwater	Glide	944	5.46%	3	1.82%	5.46%
		Pool	0	0.00%	6	0.00%	0.00%
		Riffle	201	1.17%	2	0.58%	1.17%
		Run	6,472	37.47%	9	4.16%	37.47%
Sub-reach 3 (RM 43.1–39.5) Total			**17,275**	**100.00%**	**40**		**100.00%**
4	Bar Complex	Glide	1,295	2.31%	2	1.15%	2.31%
		Pool	6,810	12.13%	5	2.43%	12.13%
		Riffle	10,197	18.16%	7	2.59%	18.16%
		Run	12,615	22.46%	6	3.74%	22.46%
	Flatwater	Glide	591	1.05%	3	0.35%	1.05%
		Pool	10,655	18.97%	6	3.16%	18.97%
		Riffle	1,278	2.27%	2	1.14%	2.27%
		Run	12,724	22.65%	9	2.52%	22.65%
Sub-reach 4 (RM 39.5–21.9) Total			**56,165**	**100.00%**	**40**		**100.00%**

Appendix C

Effective Weighted Usable Area Results by Sub-reach in the lower Tuolumne River during summer (1970–2012) using MWAT thresholds of 18-24°C

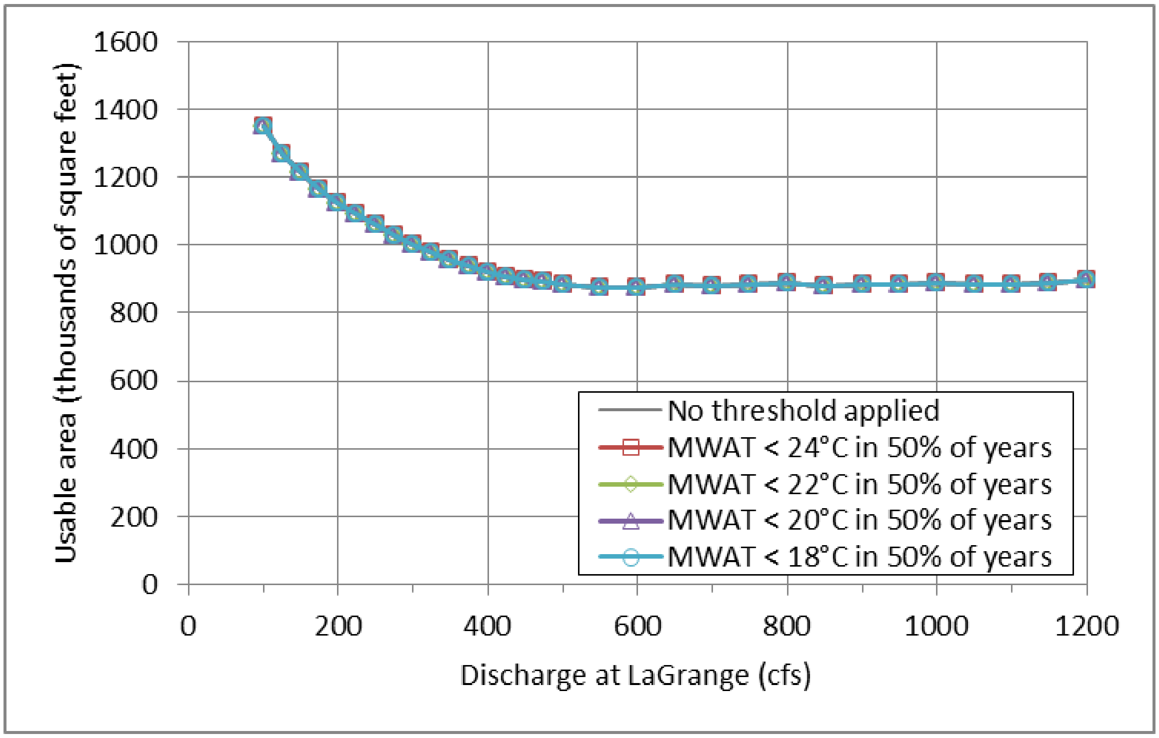

Figure C-1. Effective habitat for *O. mykiss* fry in June for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

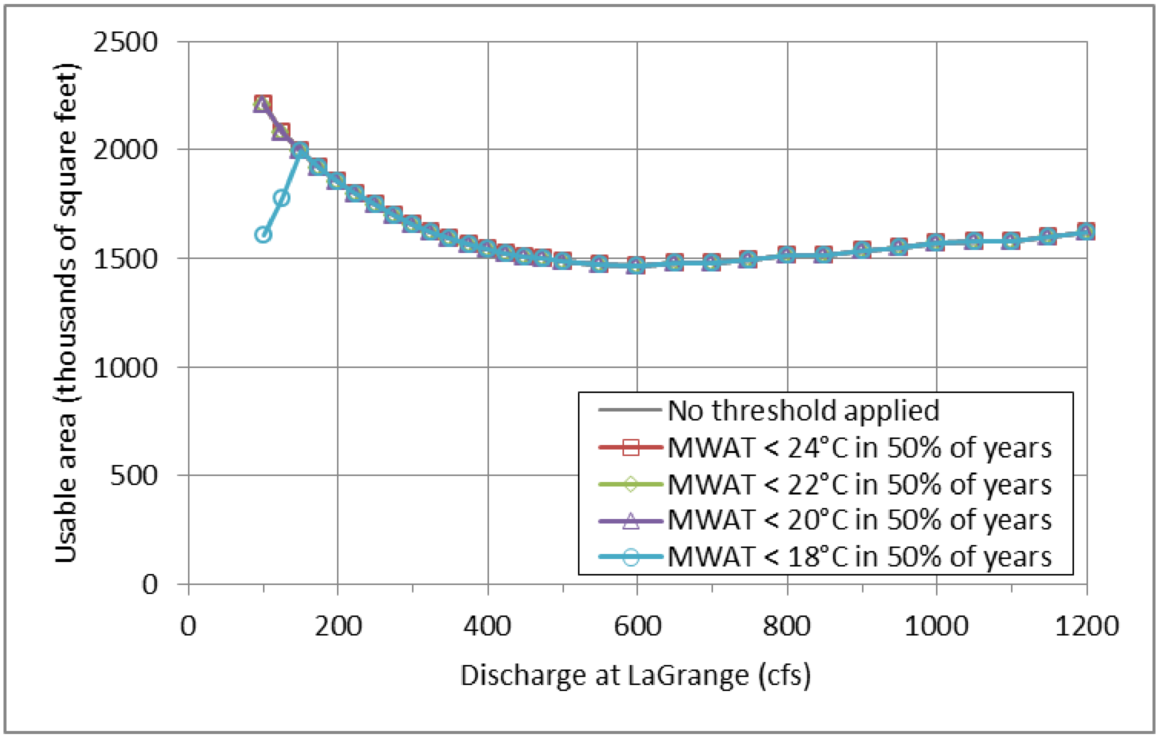

Figure C-2. Effective habitat for *O. mykiss* fry in June for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

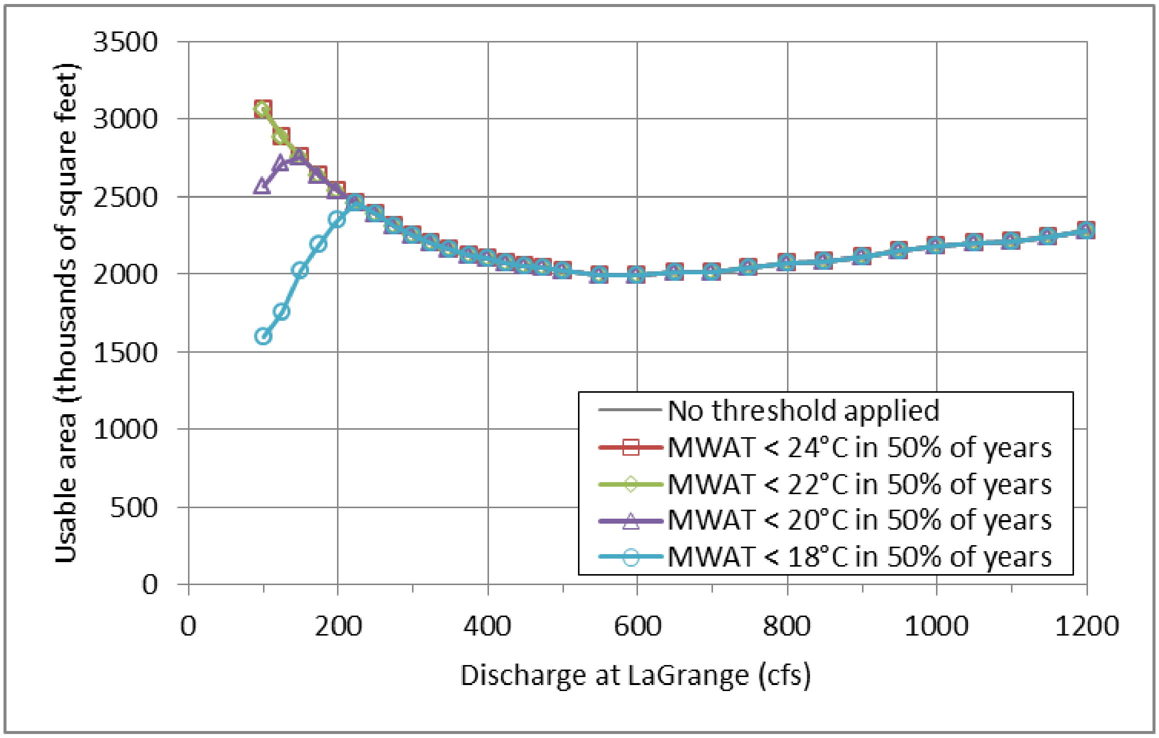

Figure C-3. Effective habitat for *O. mykiss* fry in June for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

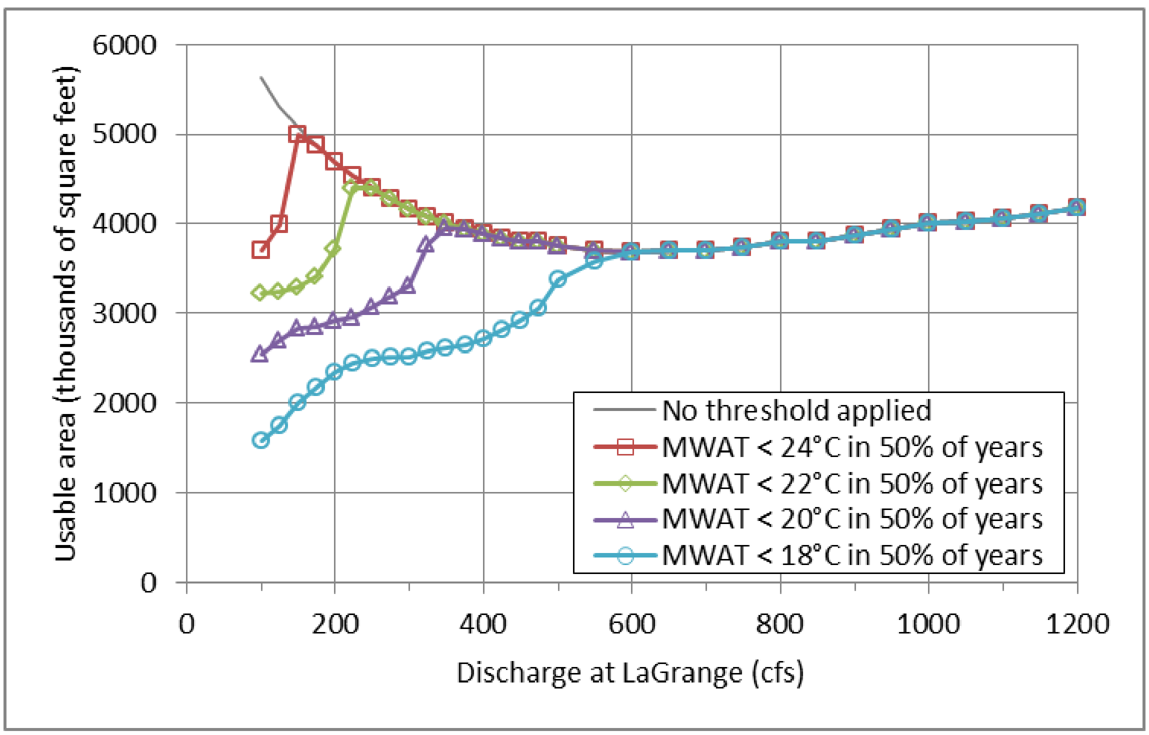

Figure C-4. Effective habitat for *O. mykiss* fry in June for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

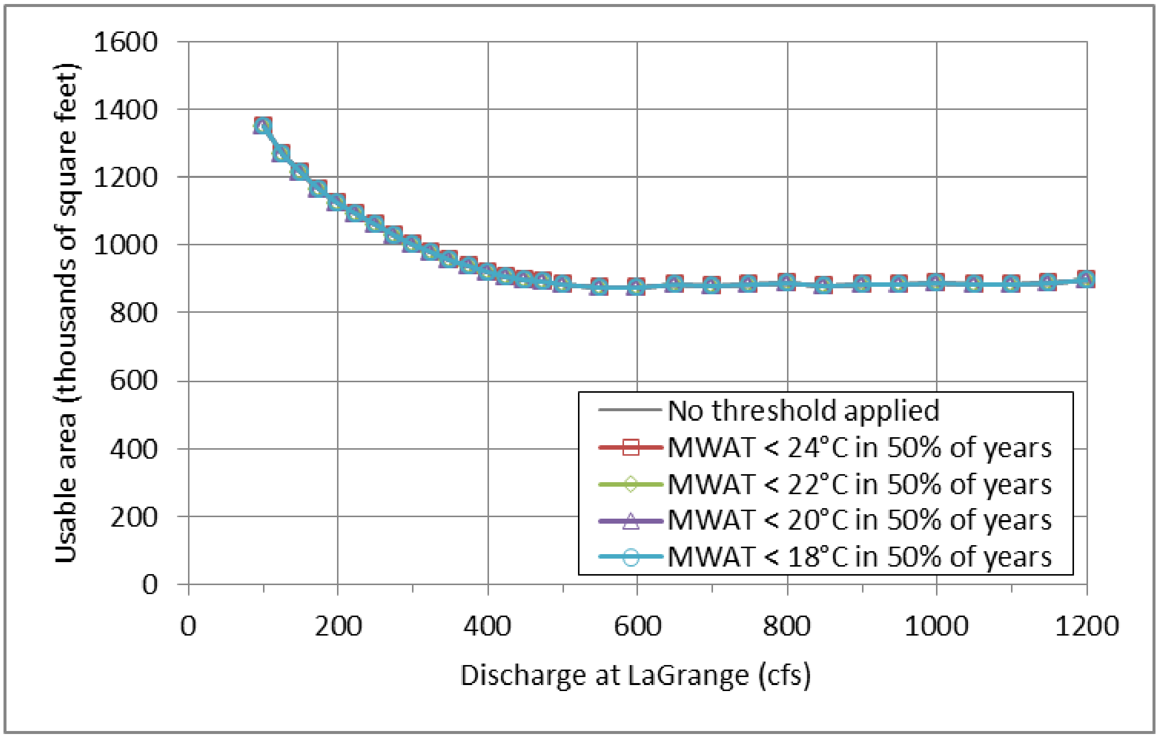

Figure C-5. Effective habitat for *O. mykiss* fry in July for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

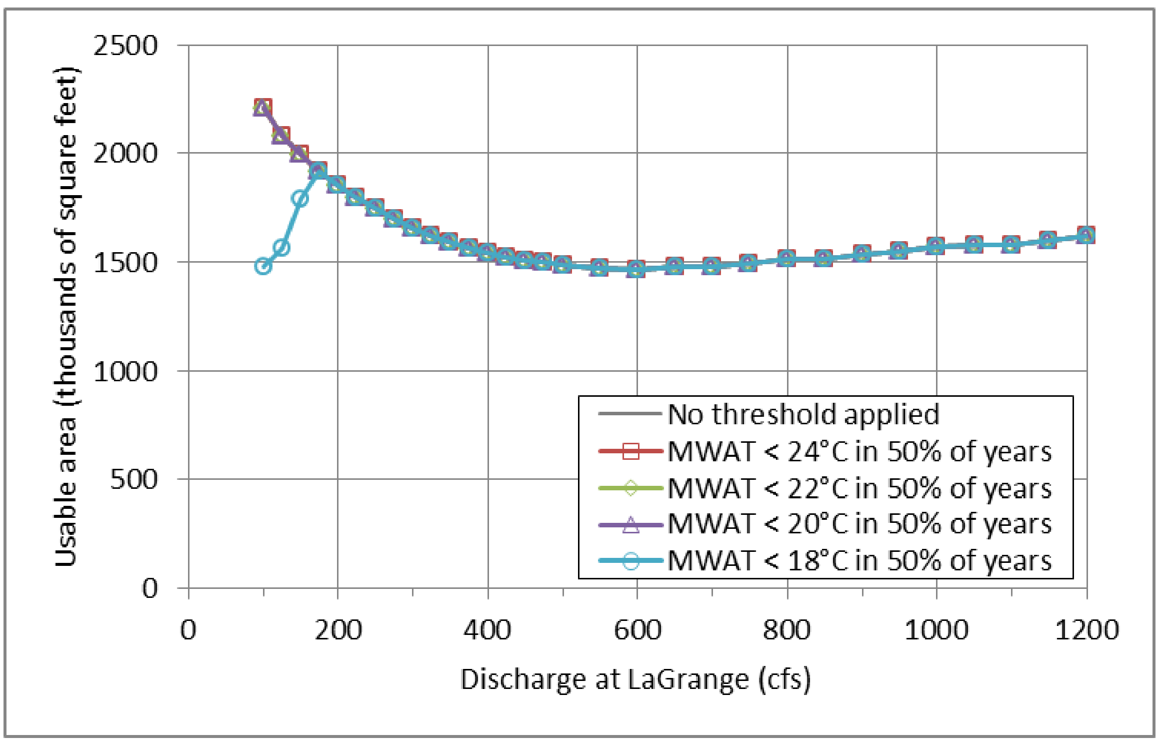

Figure C-6. Effective habitat for *O. mykiss* fry in July for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

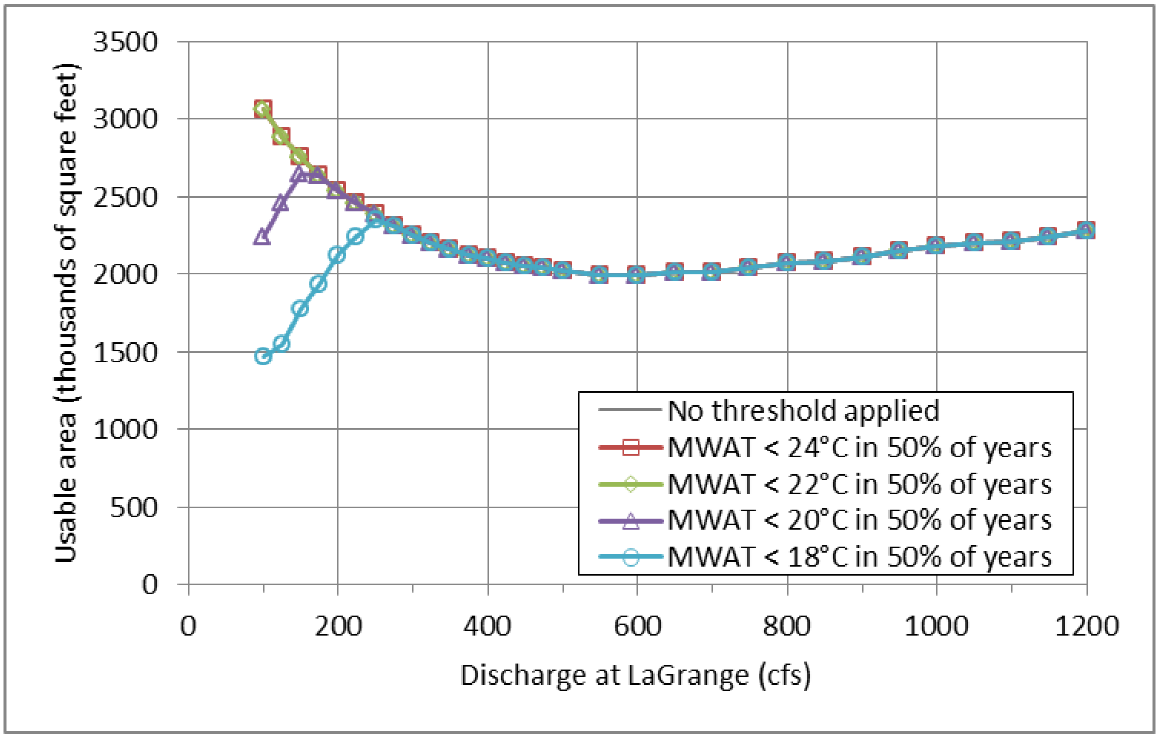

Figure C-7. Effective habitat for *O. mykiss* fry in July for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

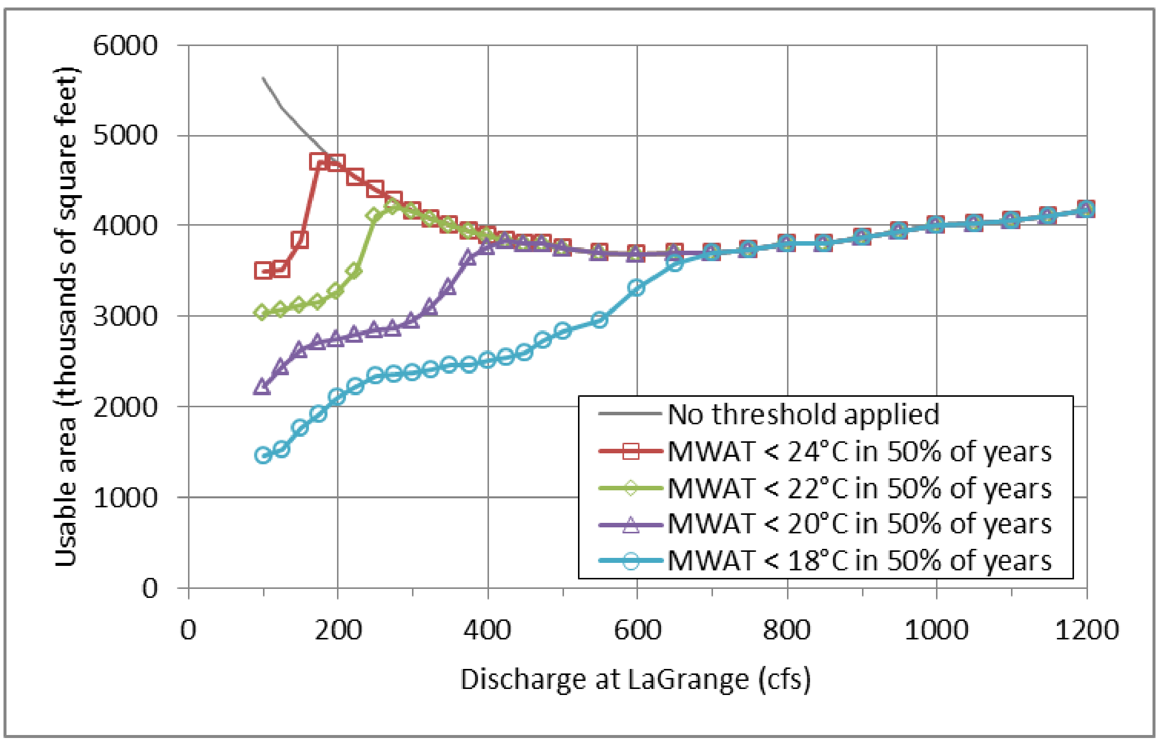

Figure C-8. Effective habitat for *O. mykiss* fry in July for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

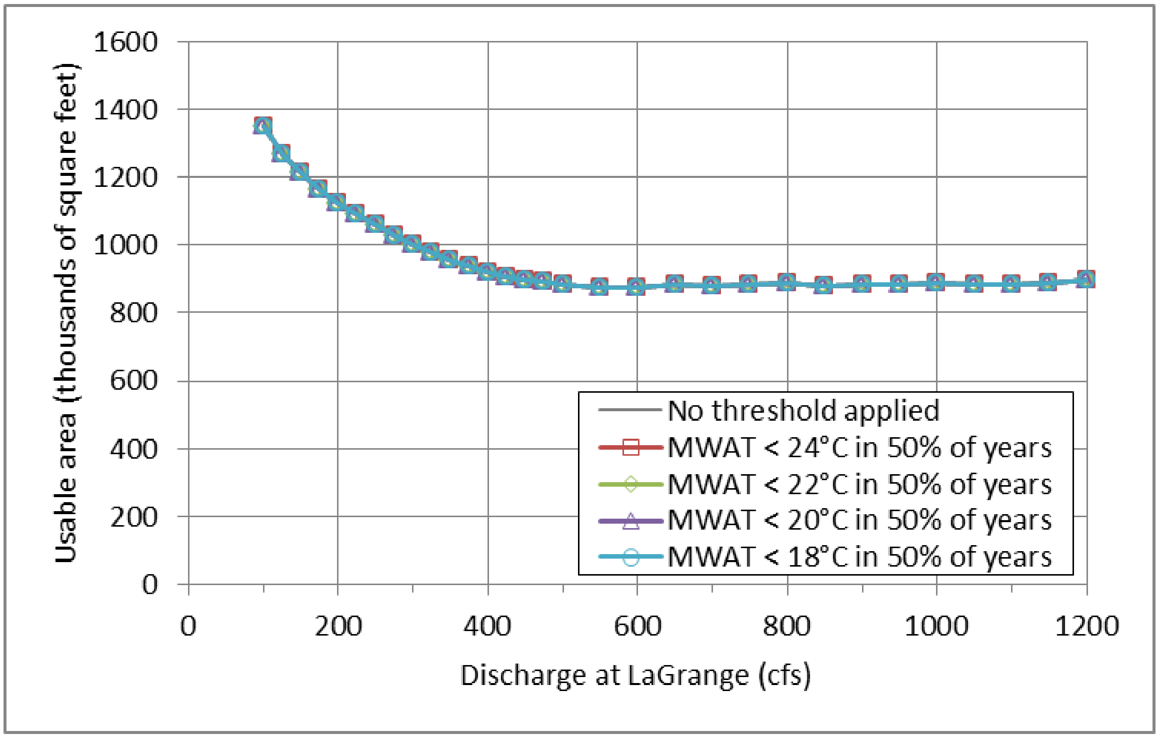

Figure C-9. Effective habitat for *O. mykiss* fry in August for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

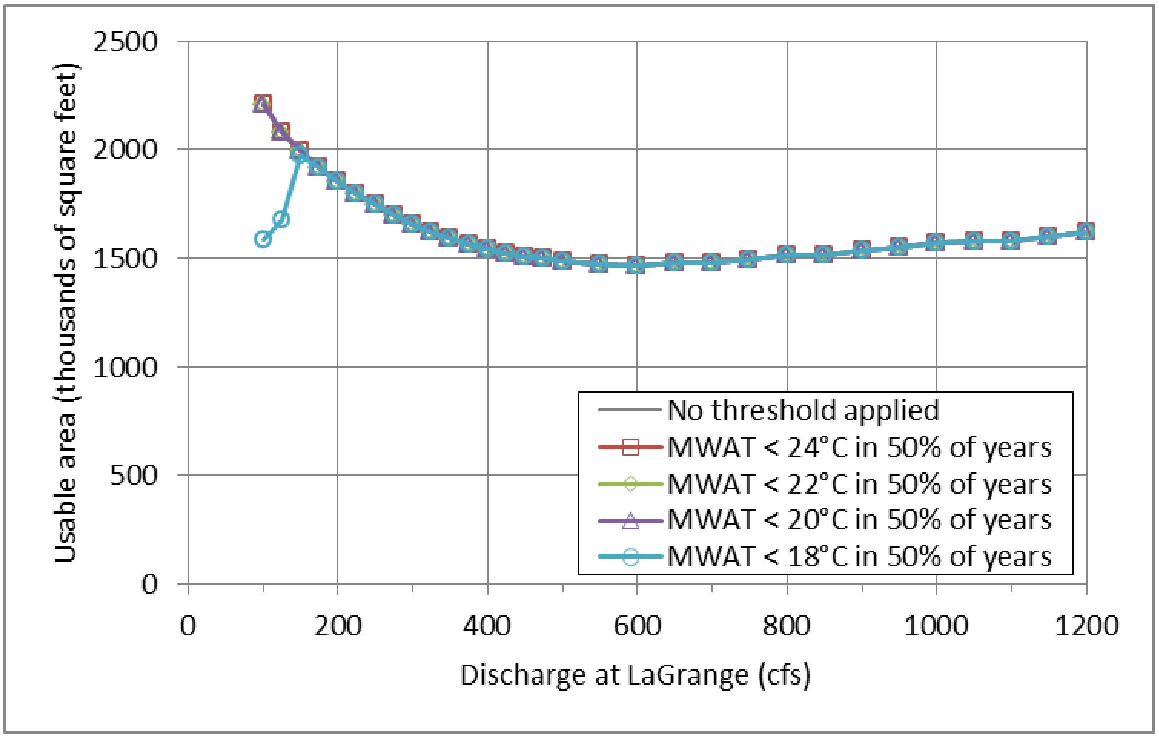

Figure C-10. Effective habitat for *O. mykiss* fry in August for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

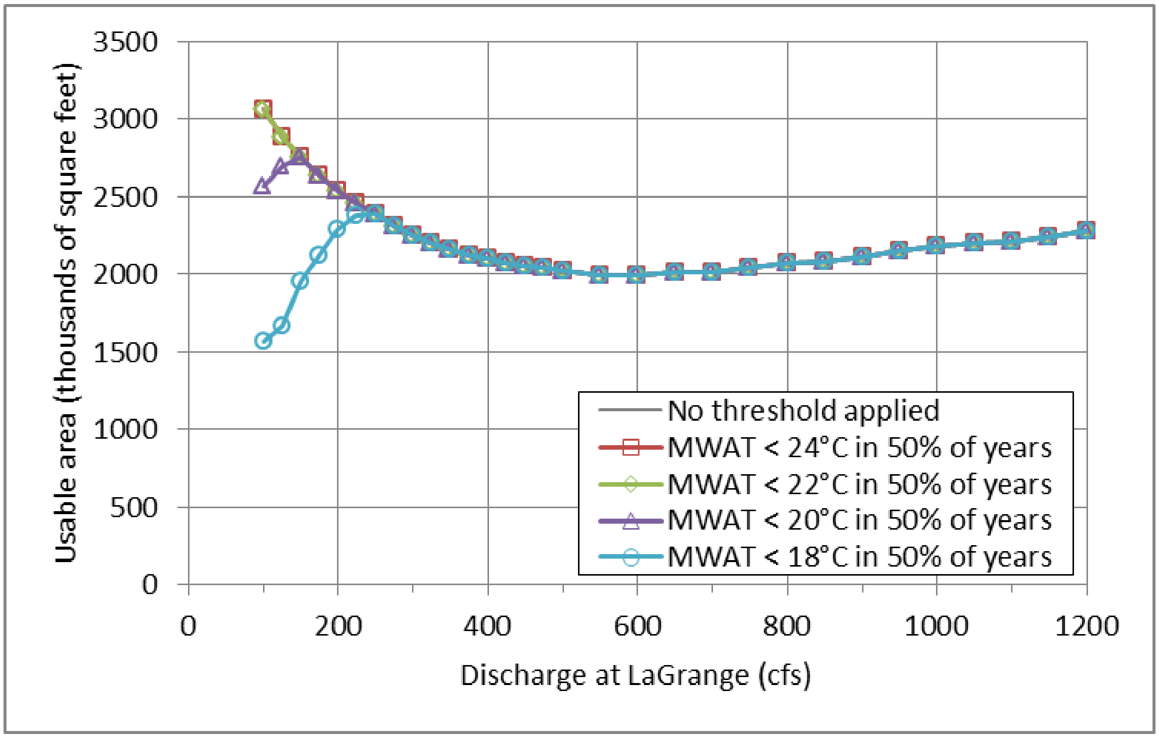

Figure C-11. Effective habitat for *O. mykiss* fry in August for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

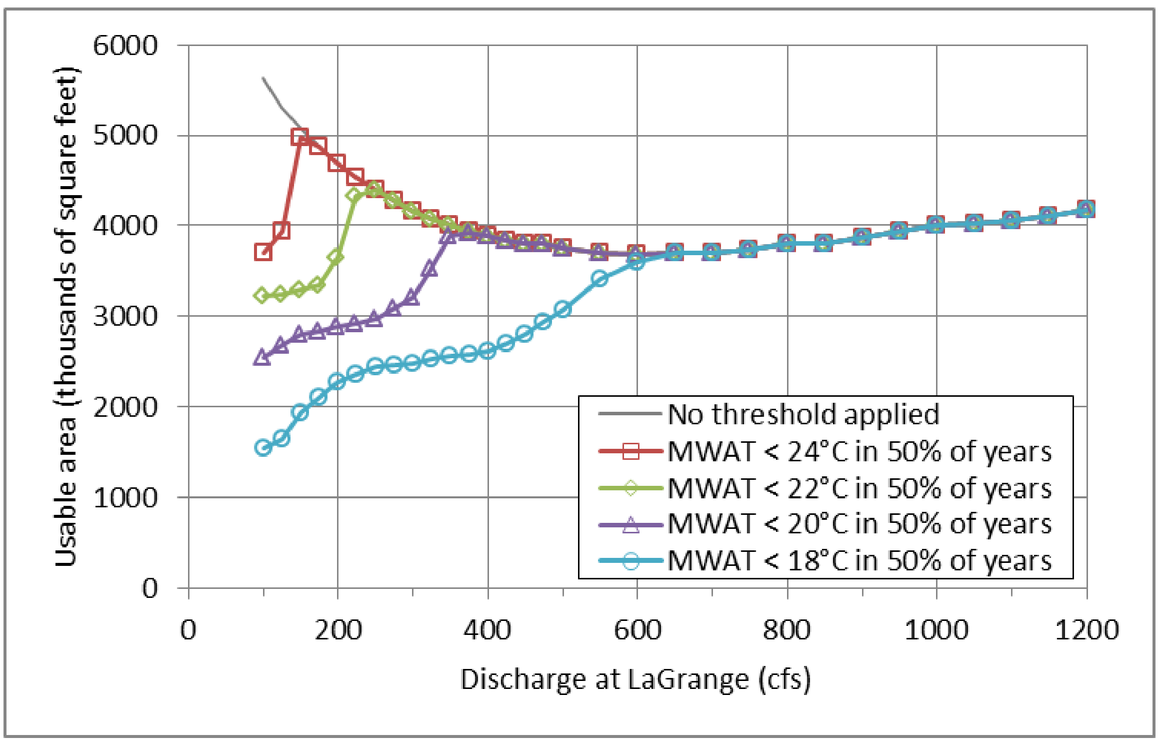

Figure C-12. Effective habitat for *O. mykiss* fry in August for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

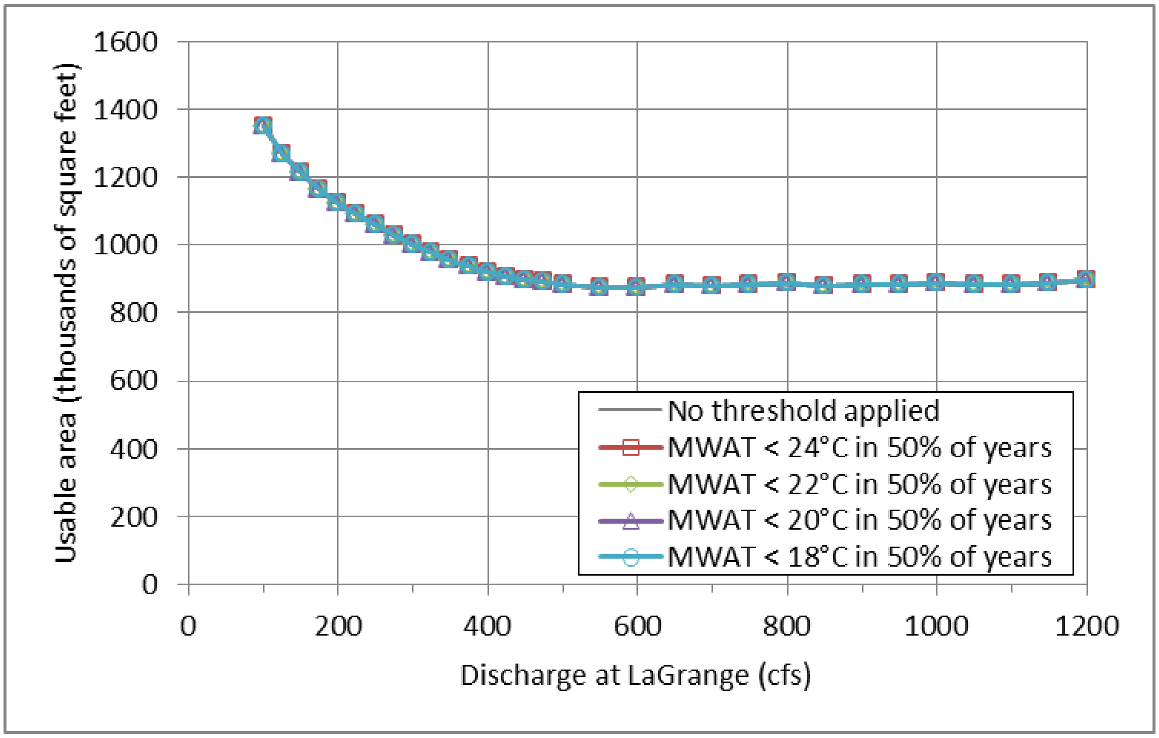

Figure C-13. Effective habitat for *O. mykiss* fry in September for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

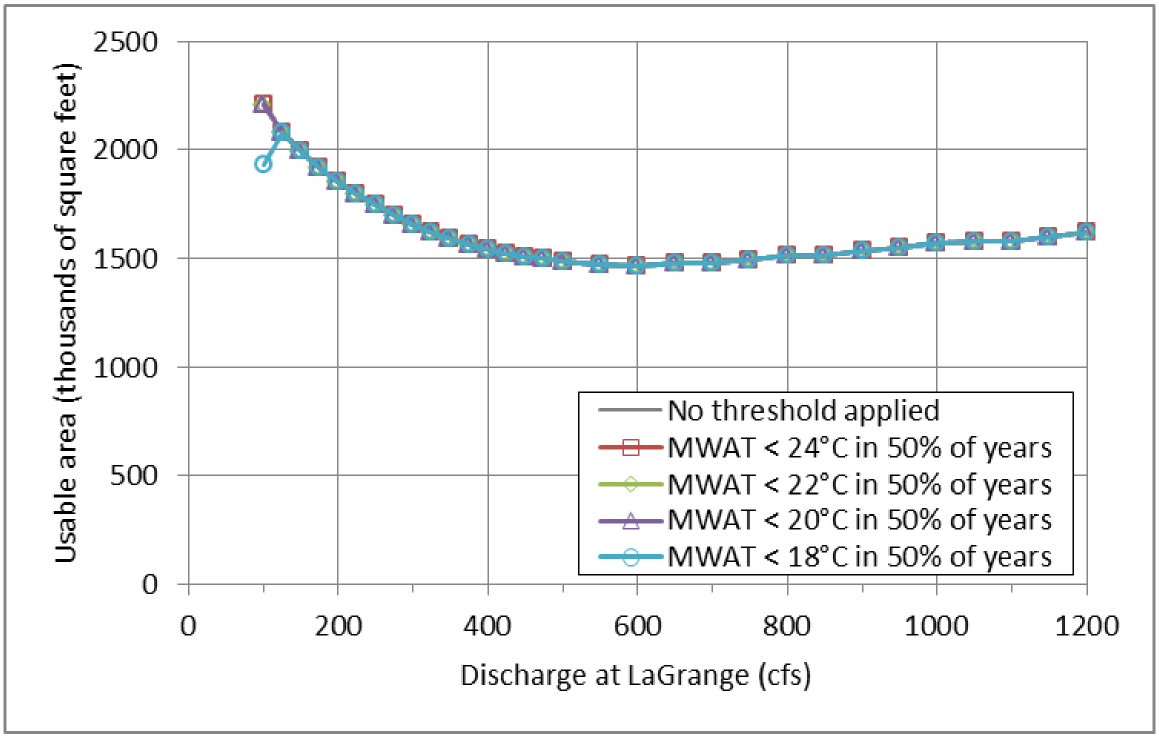

Figure C-14. Effective habitat for *O. mykiss* fry in September for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

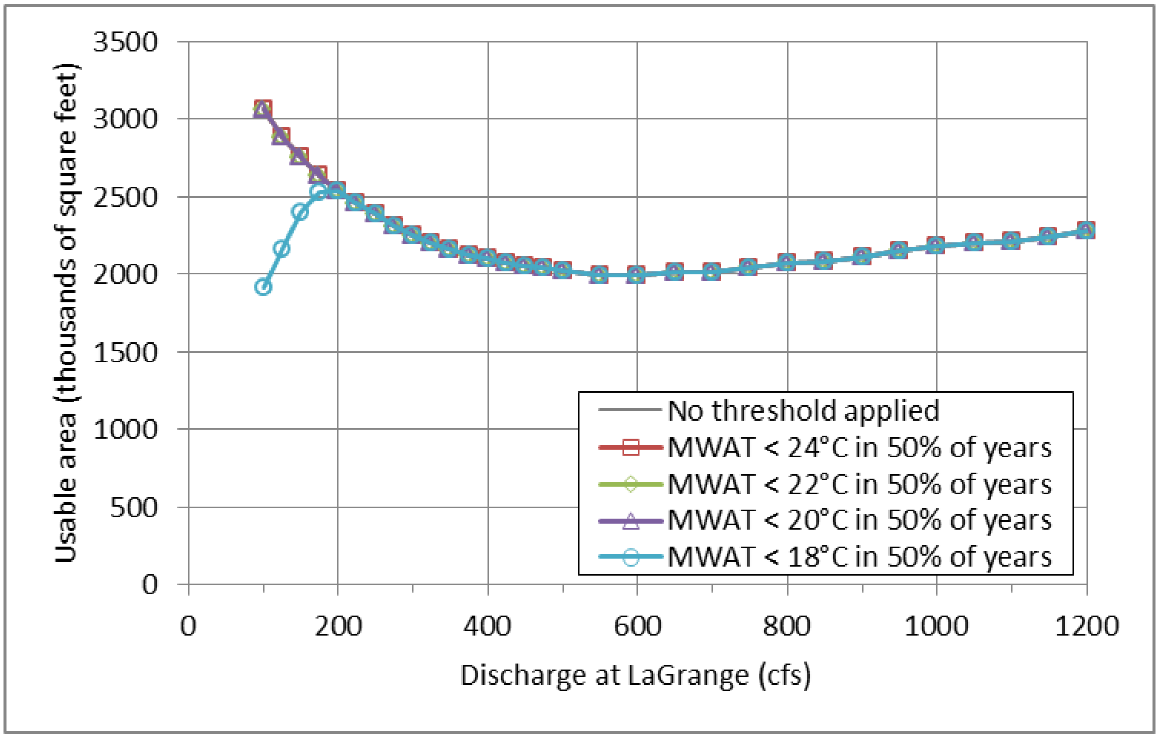

Figure C-15. Effective habitat for *O. mykiss* fry in September for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

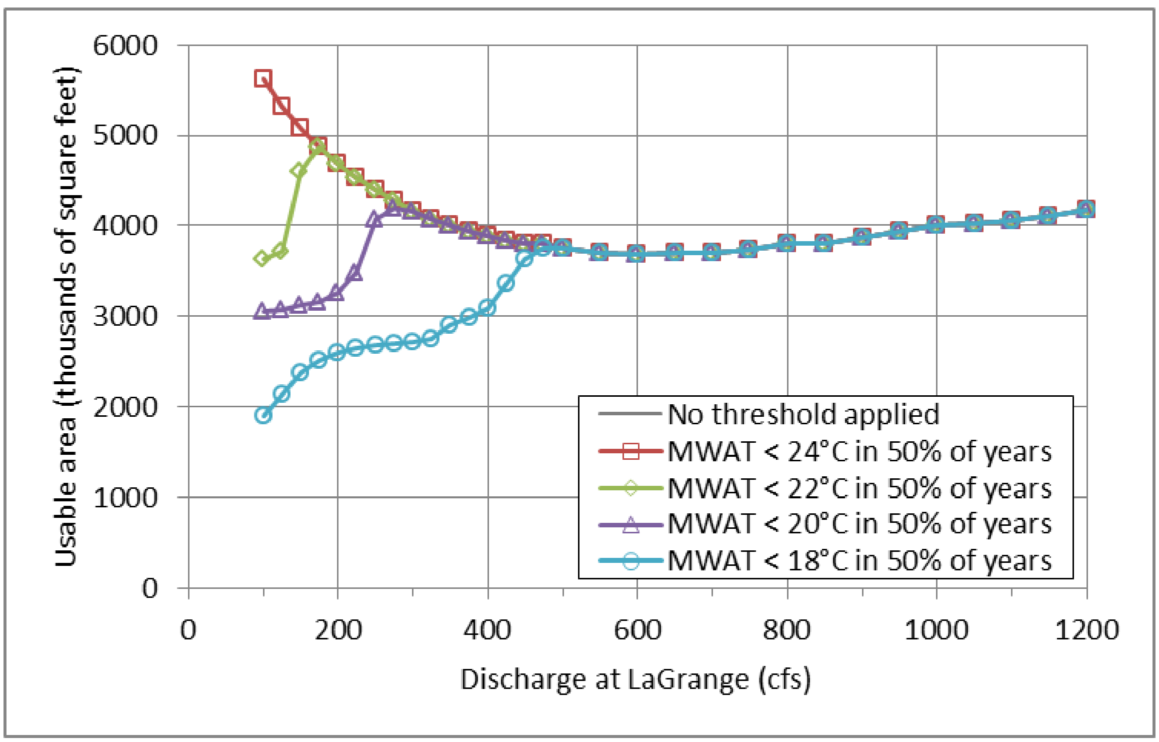

Figure C-16. Effective habitat for *O. mykiss* fry in September for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

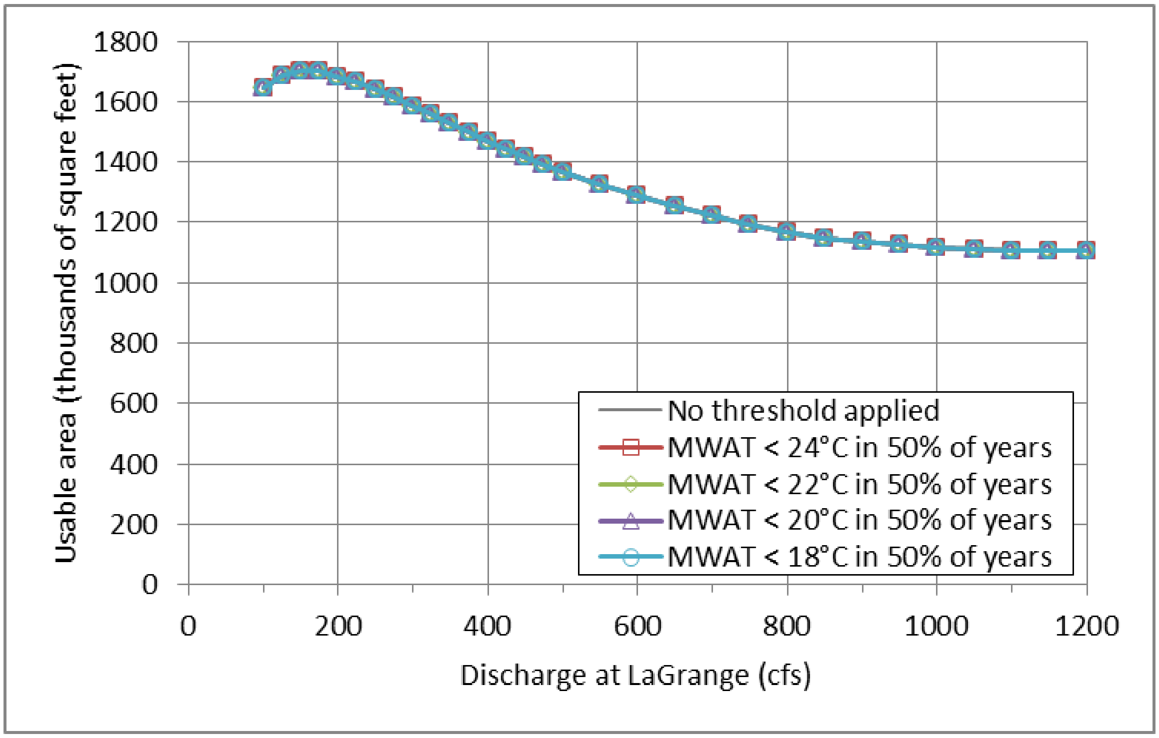

Figure C-17. Effective habitat for *O. mykiss* juvenile in June for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

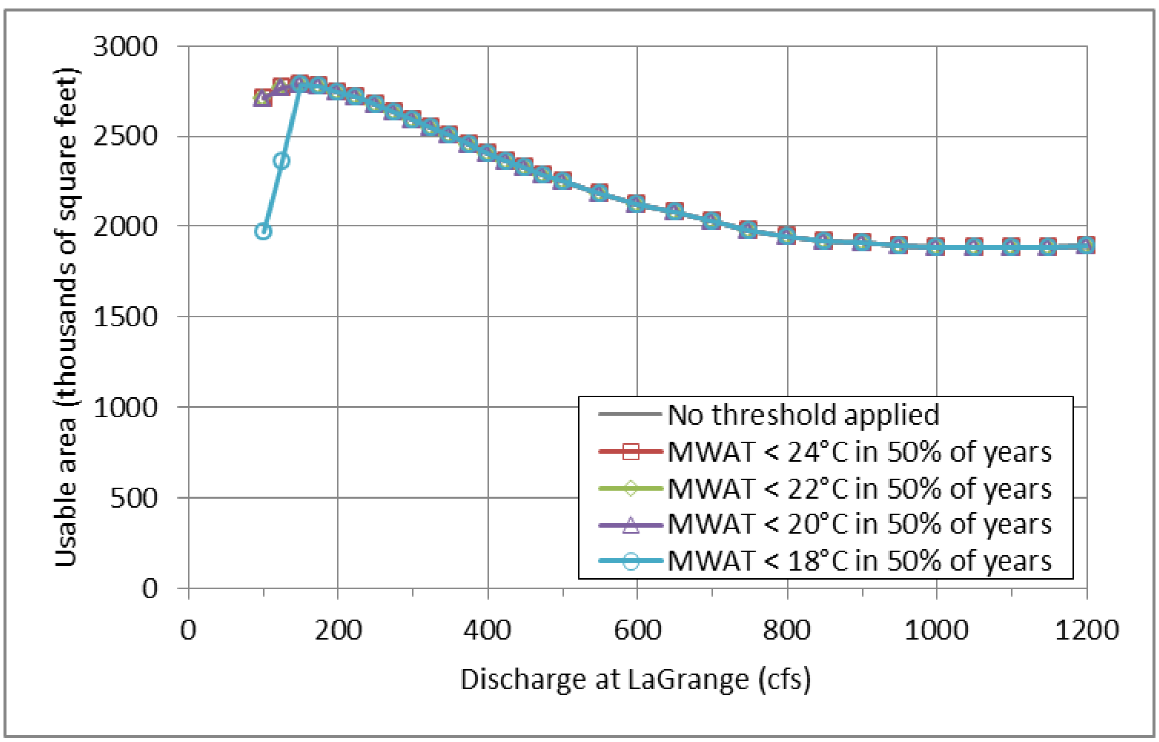

Figure C-18. Effective habitat for *O. mykiss* juvenile in June for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

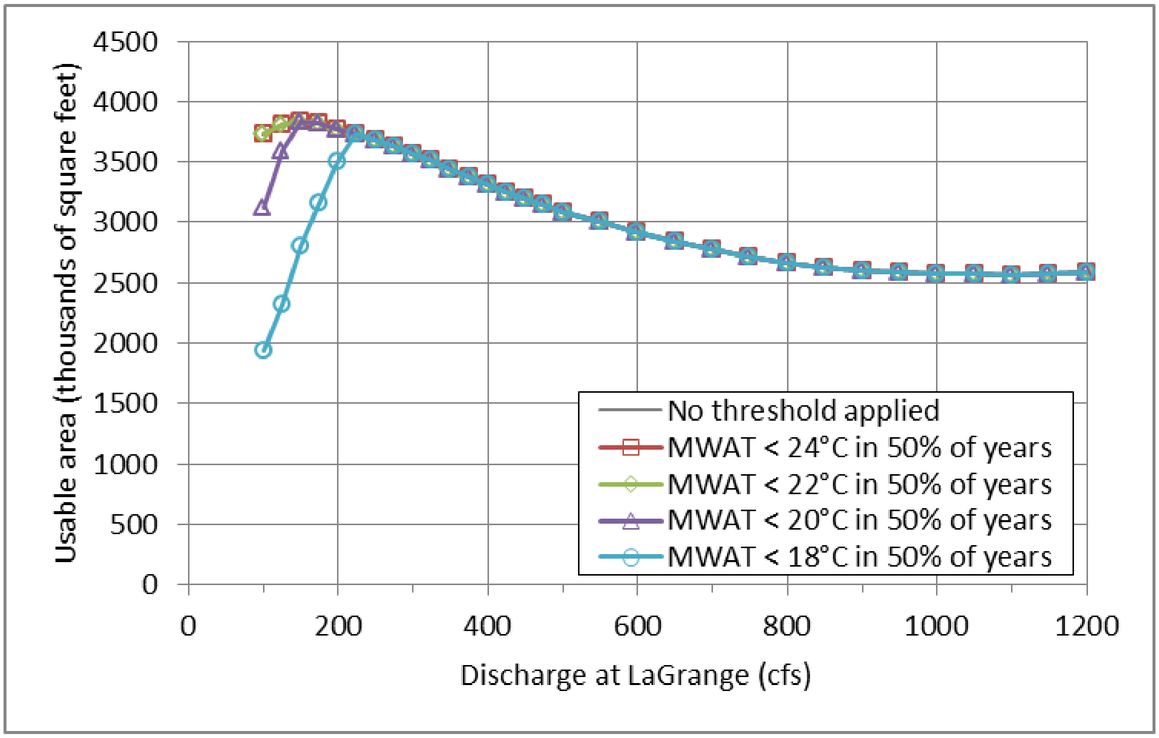

Figure C-19. Effective habitat for *O. mykiss* juvenile in June for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

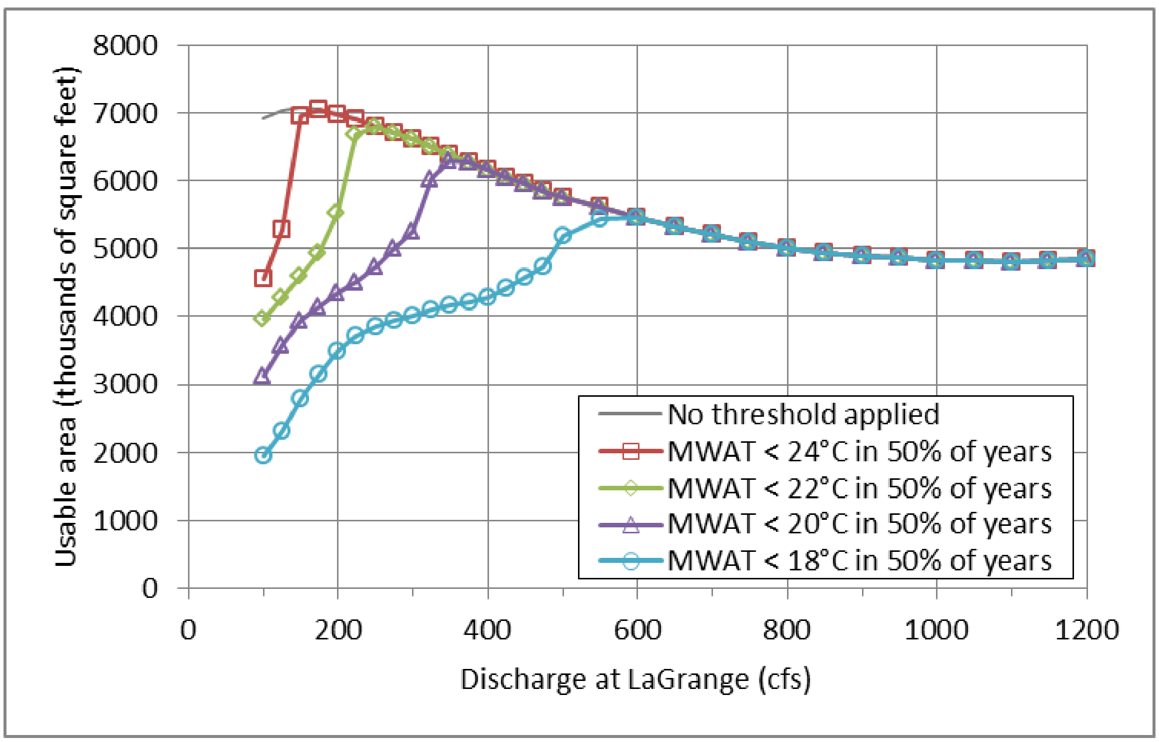

Figure C-20. Effective habitat for *O. mykiss* juvenile in June for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

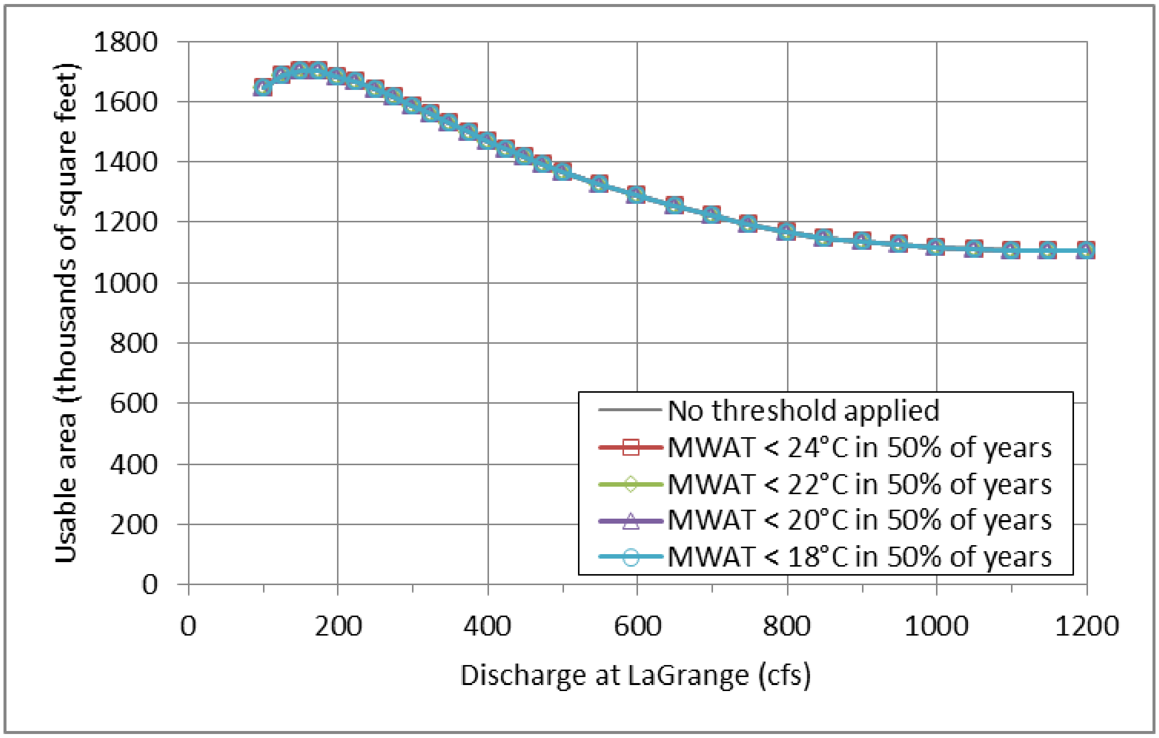

Figure C-21. Effective habitat for *O. mykiss* juvenile in July for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

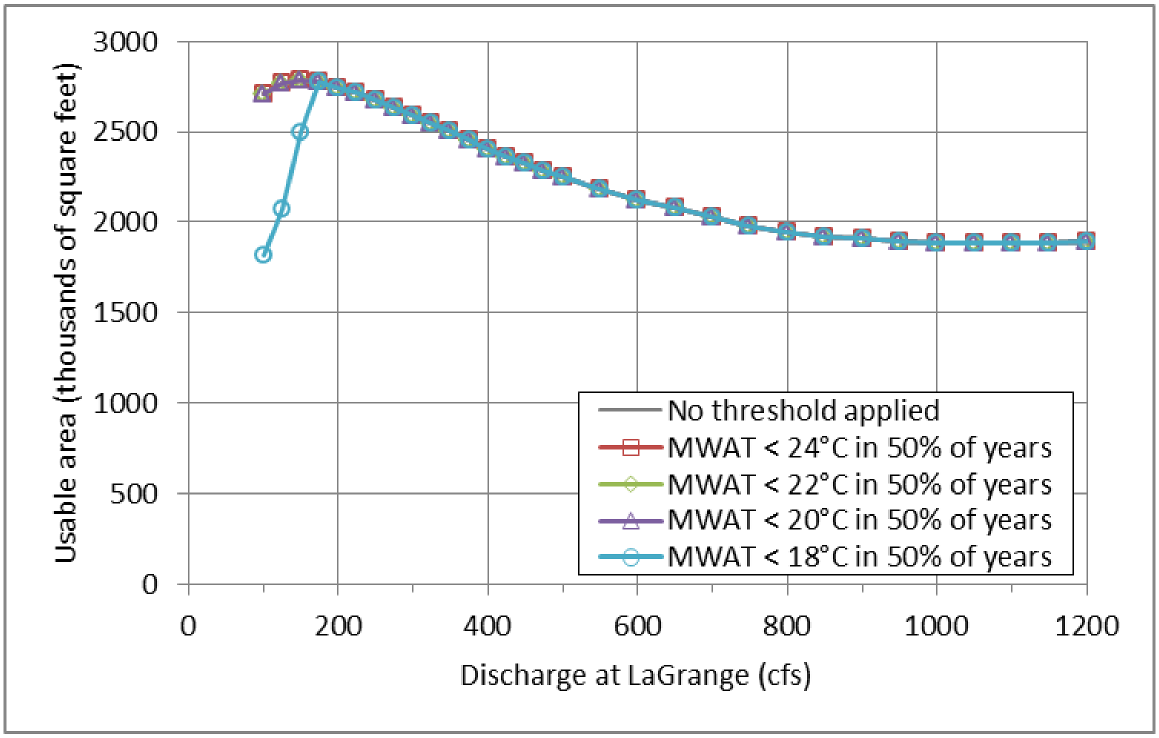

Figure C-22. Effective habitat for *O. mykiss* juvenile in July for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

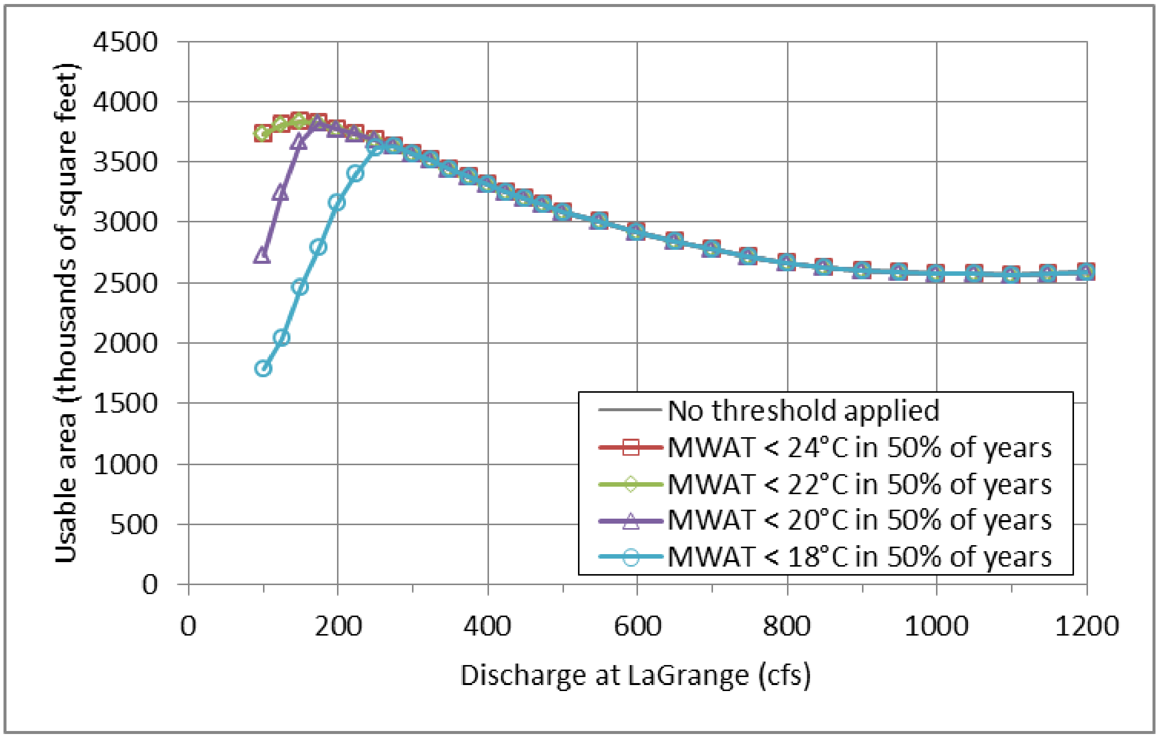

Figure C-23. Effective habitat for *O. mykiss* juvenile in July for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

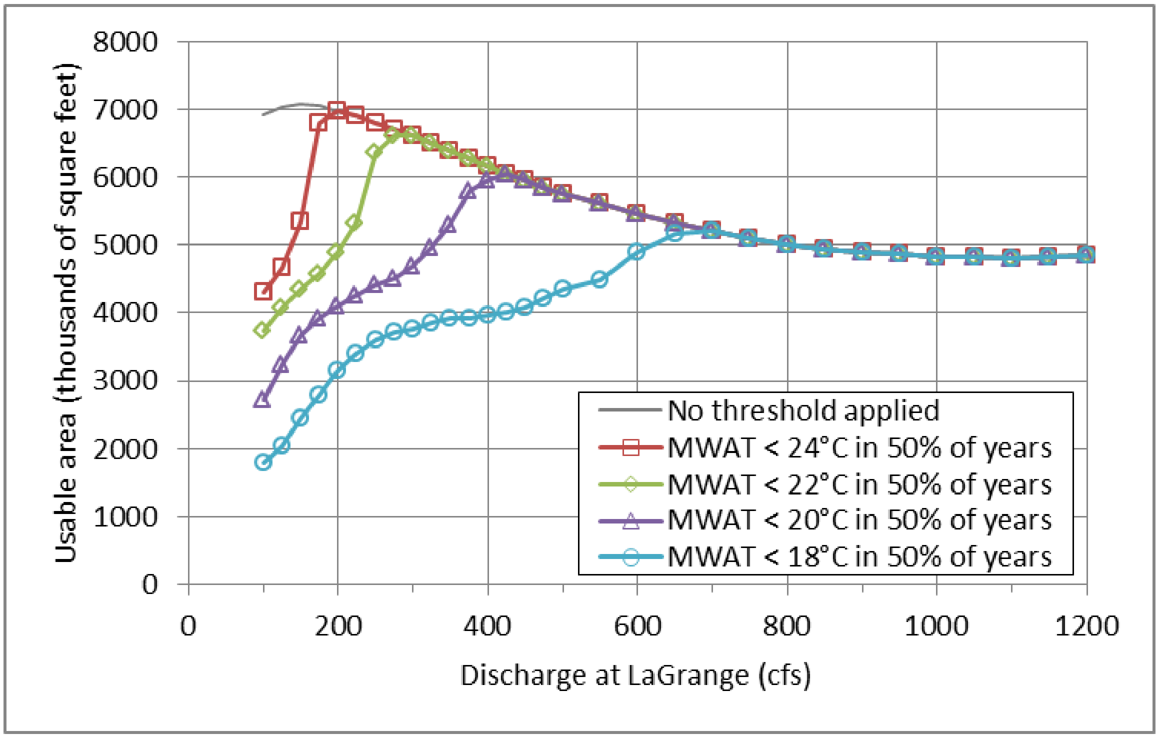

Figure C-24. Effective habitat for *O. mykiss* juvenile in July for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

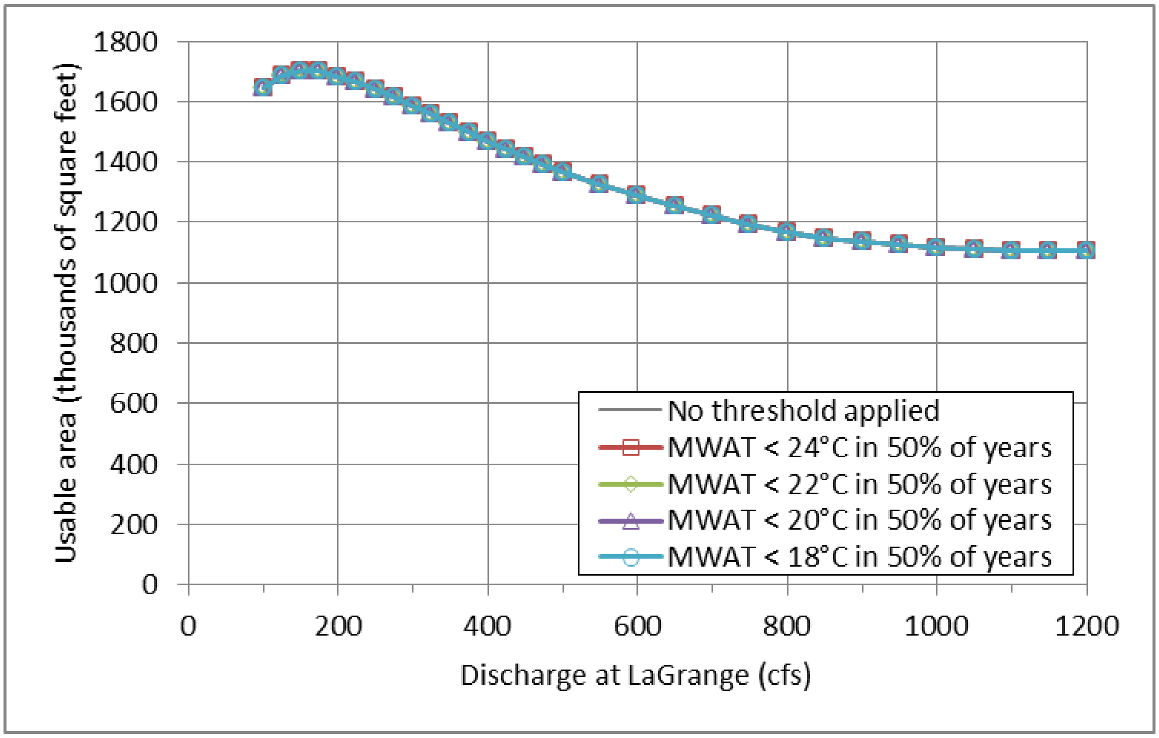

Figure C-25. Effective habitat for *O. mykiss* juvenile in August for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

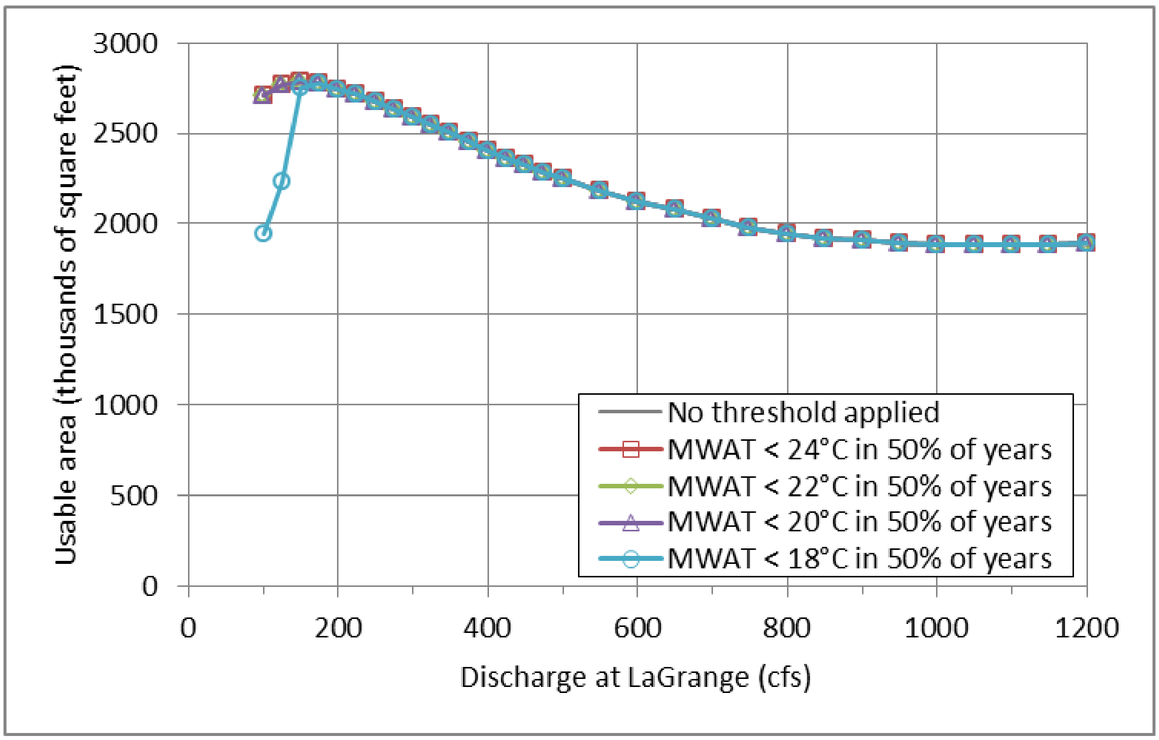

Figure C-26. Effective habitat for *O. mykiss* juvenile in August for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

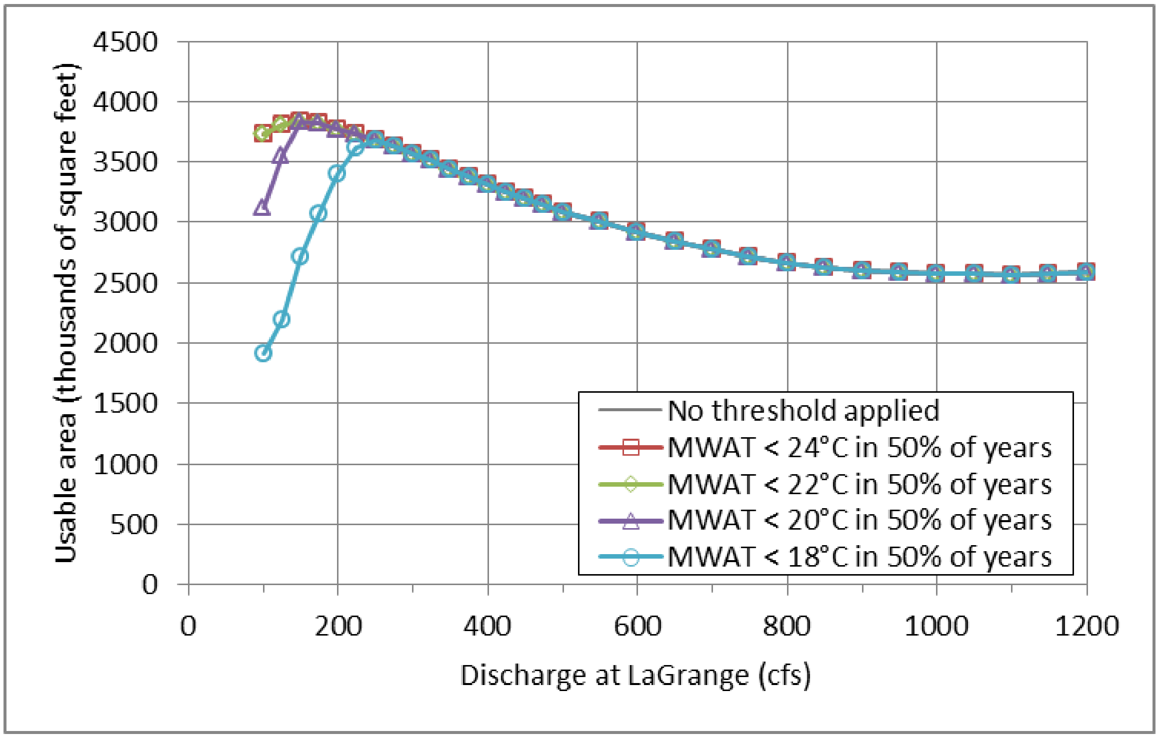

Figure C-27. Effective habitat for *O. mykiss* juvenile in August for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

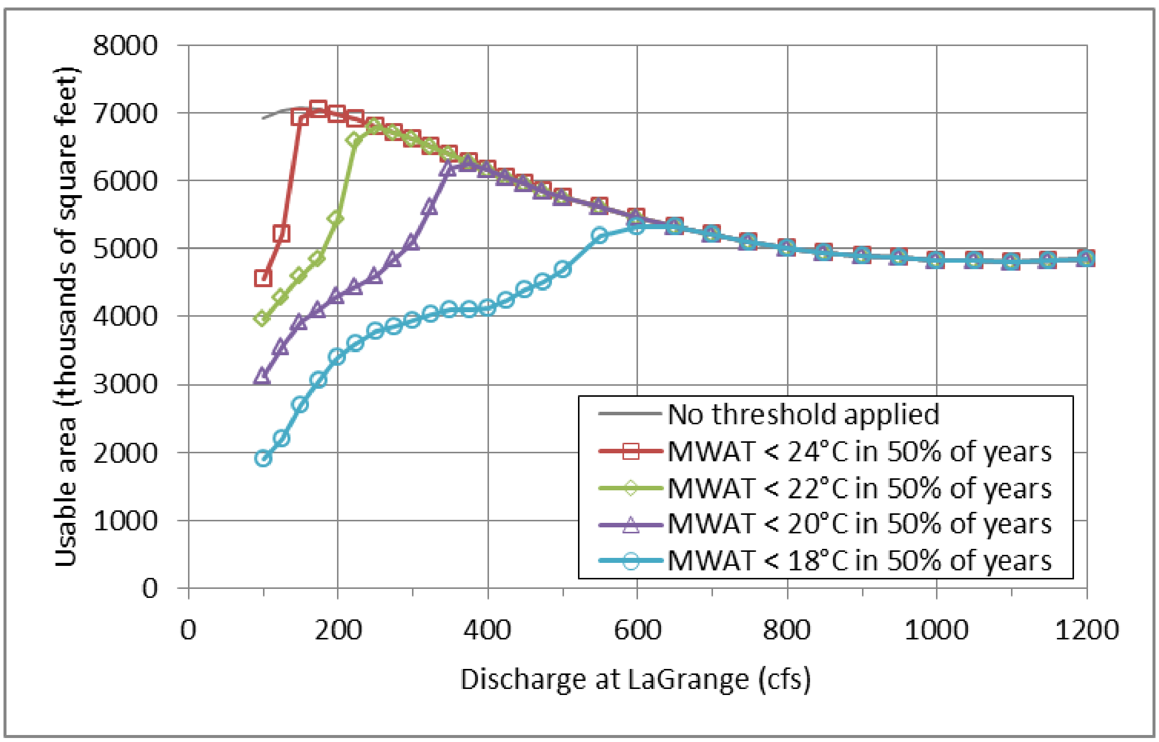

Figure C-28. Effective habitat for *O. mykiss* juvenile in August for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

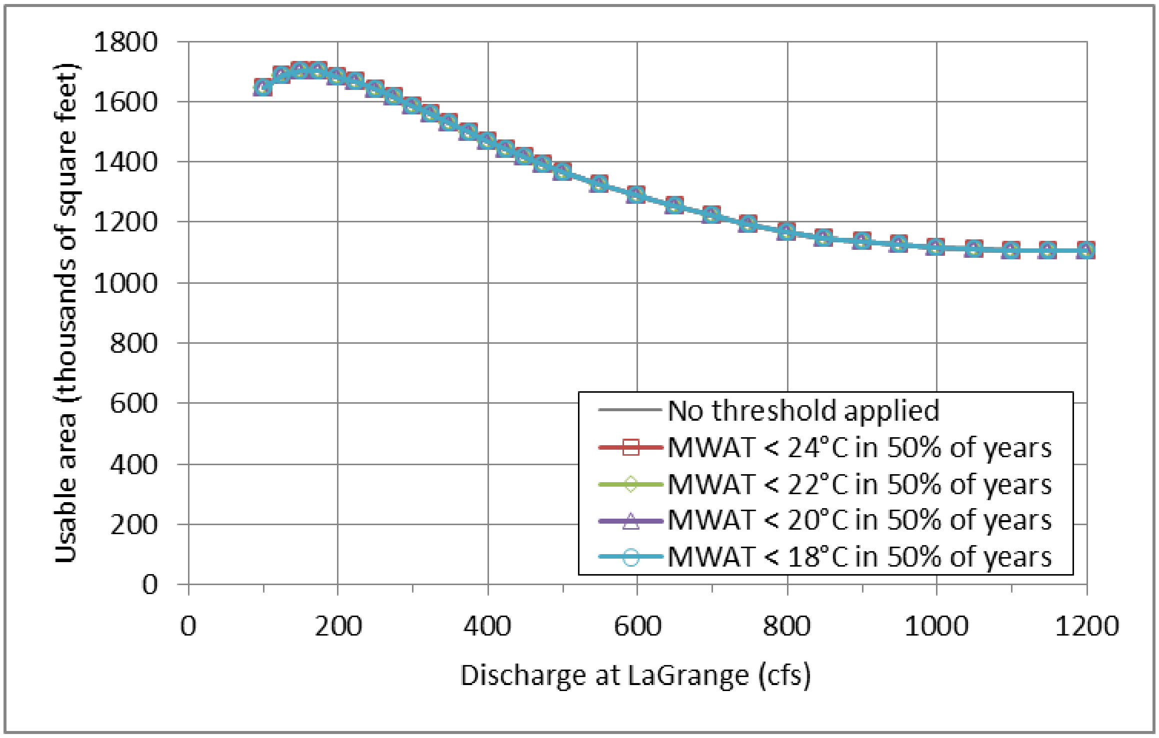

Figure C-29. Effective habitat for *O. mykiss* juvenile in September for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

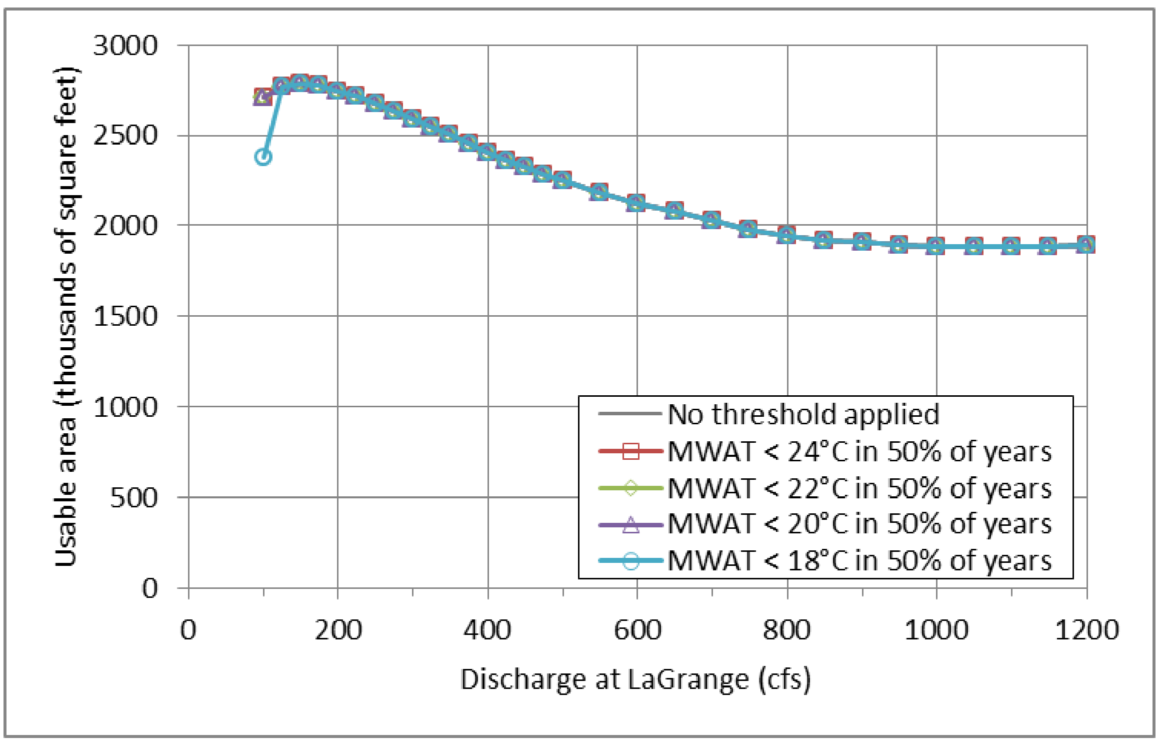

Figure C-30. Effective habitat for *O. mykiss* juvenile in September for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

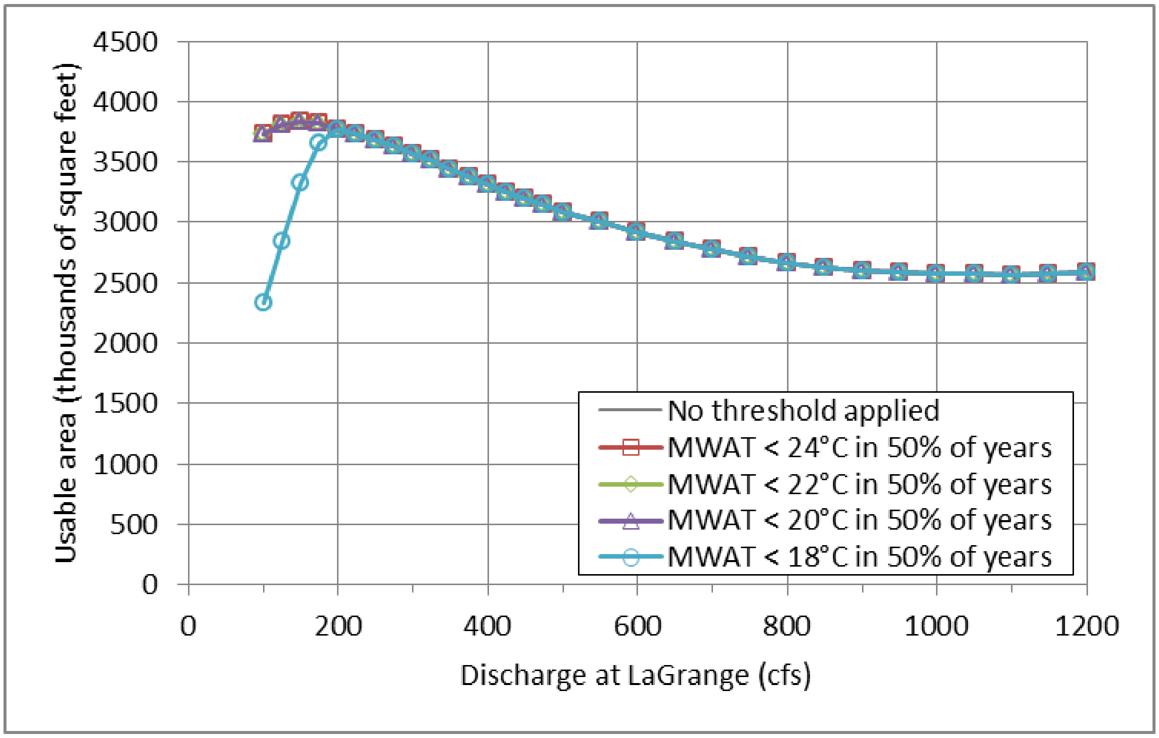

Figure C-31. Effective habitat for *O. mykiss* juvenile in September for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

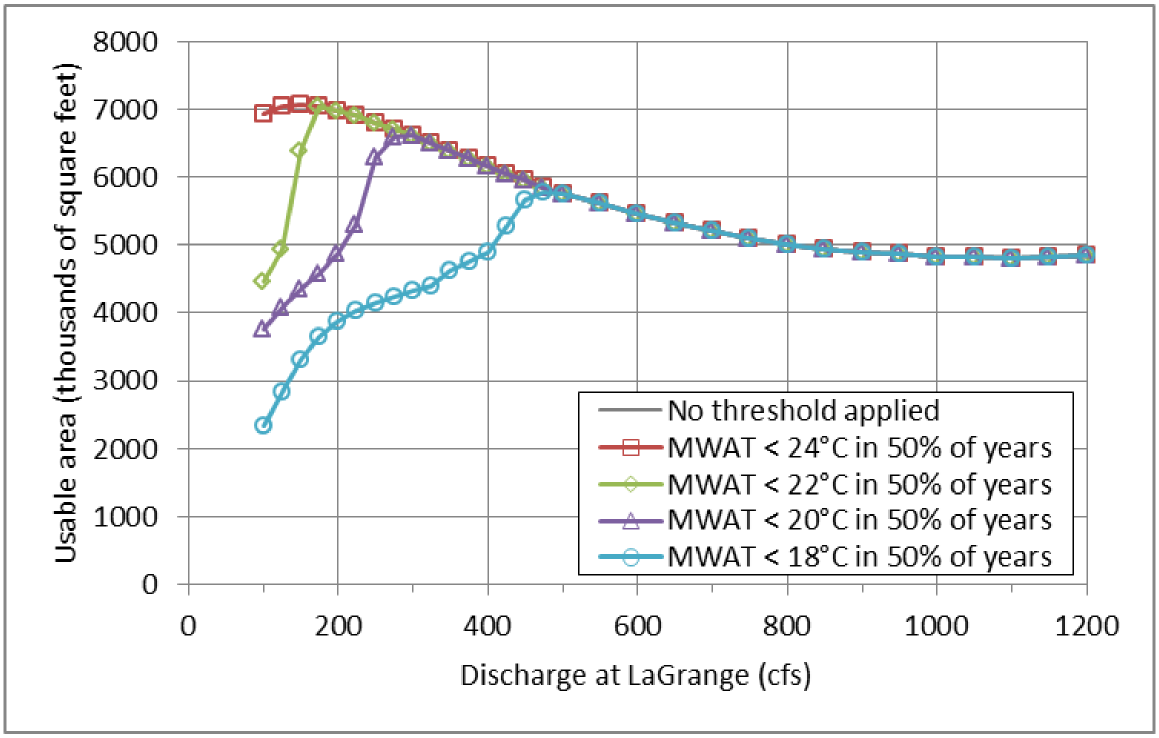

Figure C-32. Effective habitat for *O. mykiss* juvenile in September for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

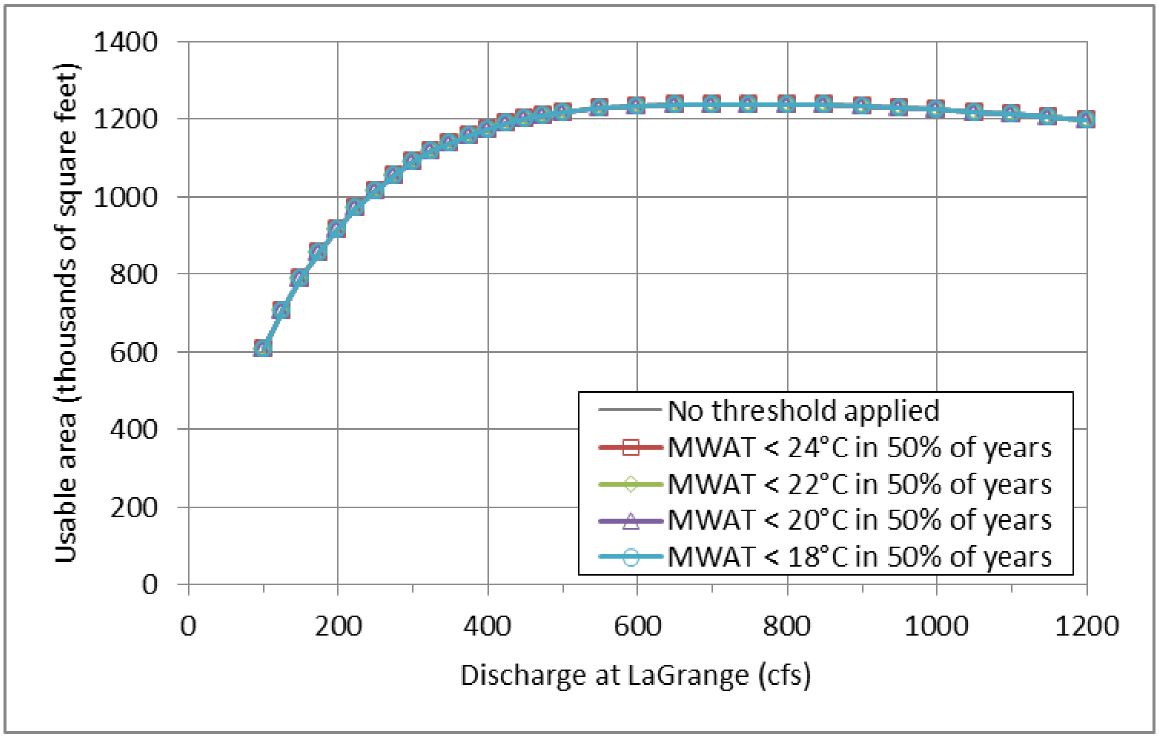

Figure C-33. Effective habitat for *O. mykiss* adult in June for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

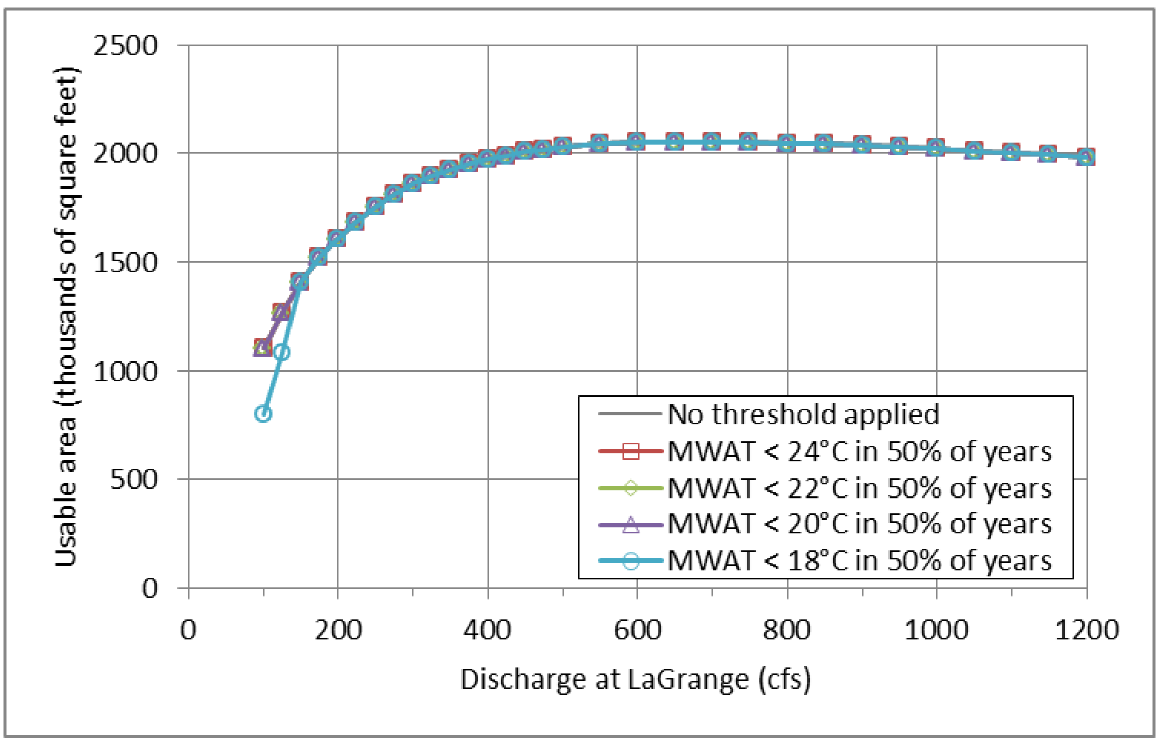

Figure C-34. Effective habitat for *O. mykiss* adult in June for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

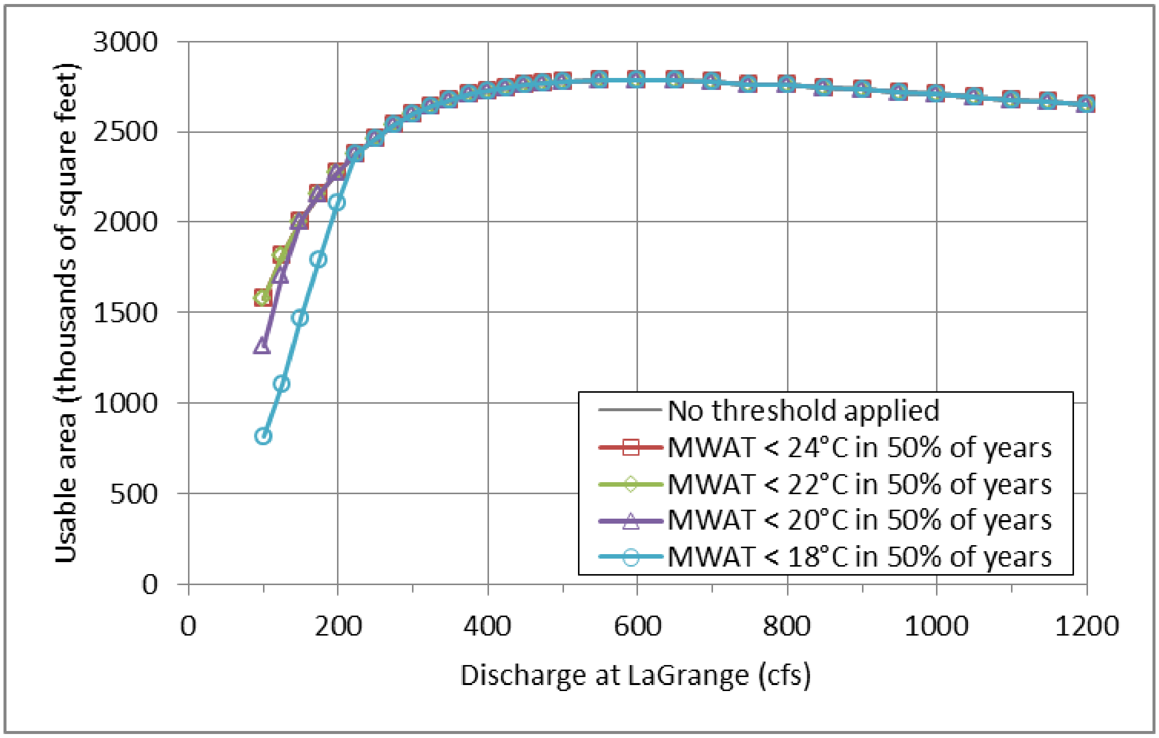

Figure C-35. Effective habitat for *O. mykiss* adult in June for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

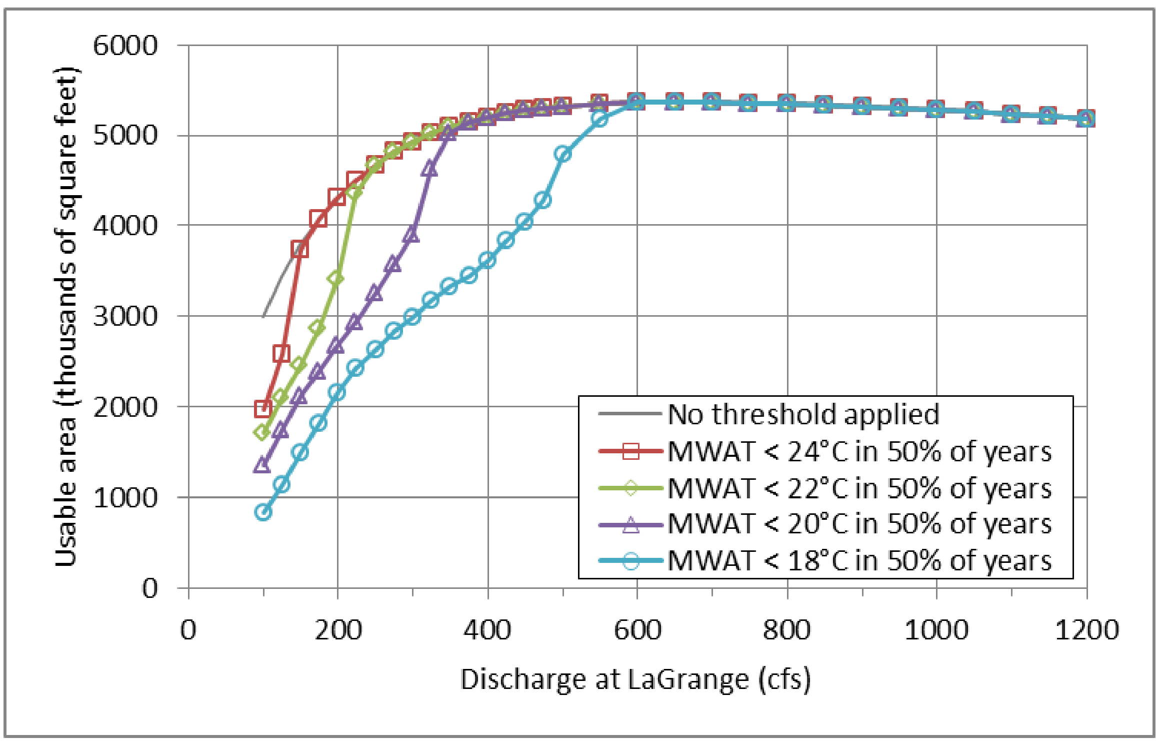

Figure C-36. Effective habitat for *O. mykiss* adult in June for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

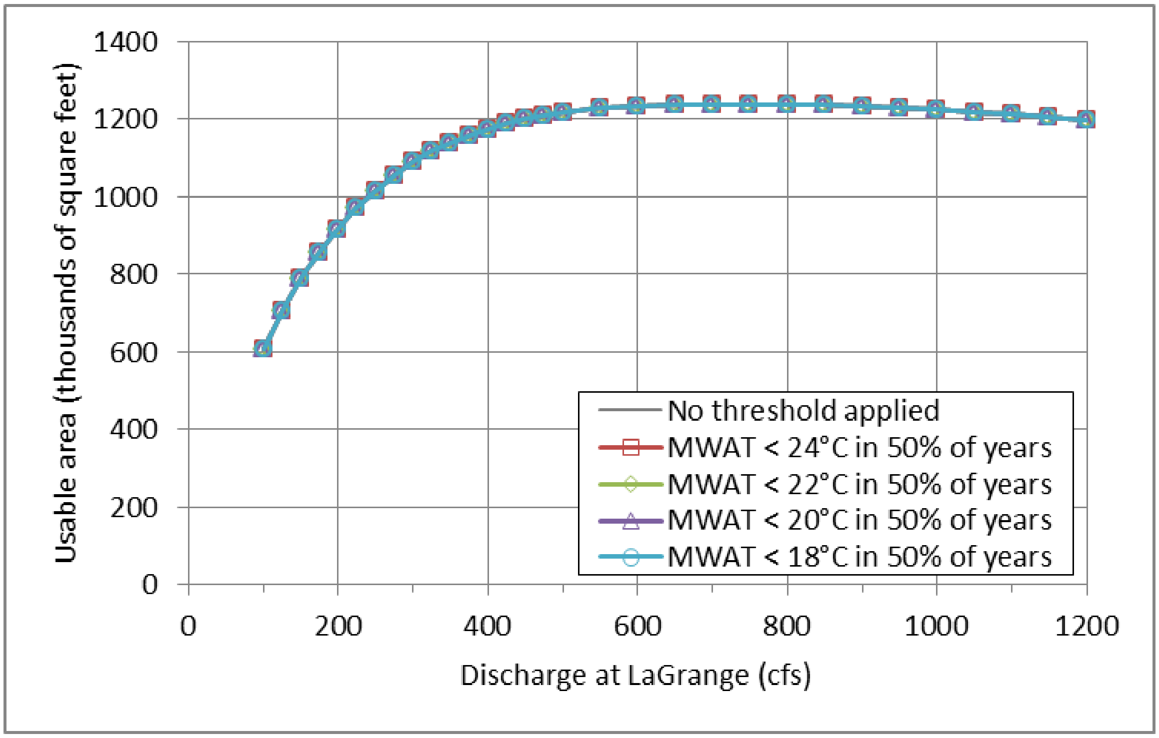

Figure C-37. Effective habitat for *O. mykiss* adult in July for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

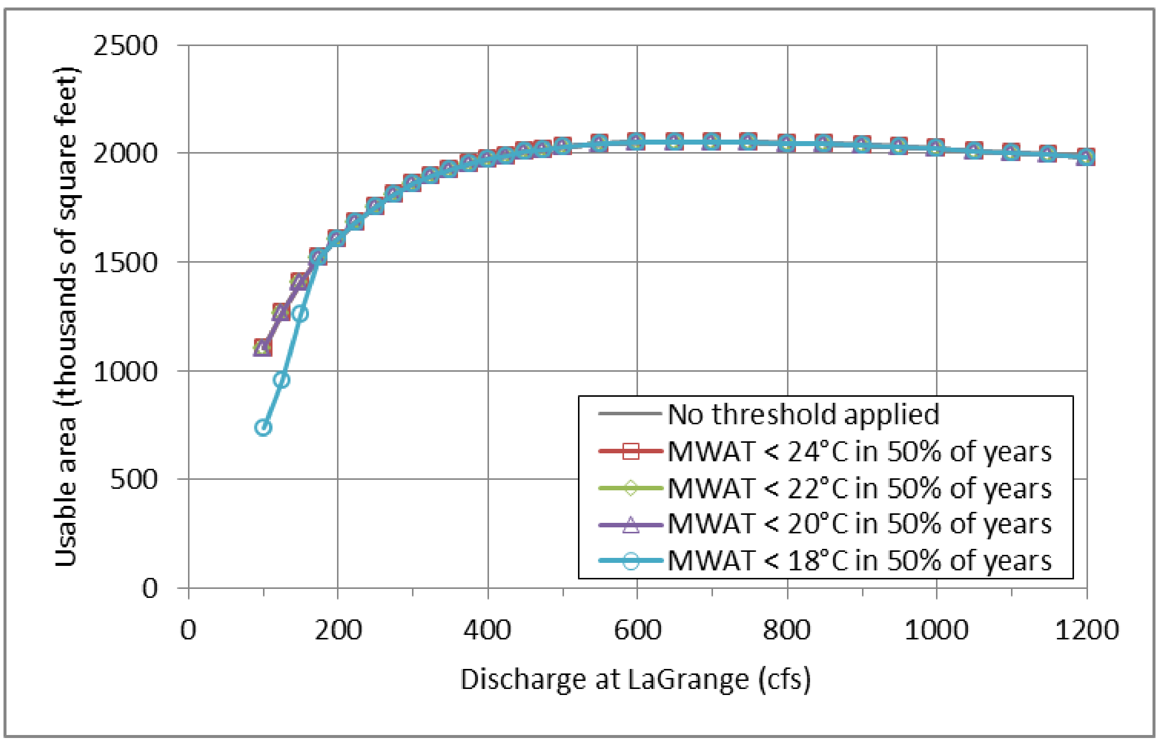

Figure C-38. Effective habitat for *O. mykiss* adult in July for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

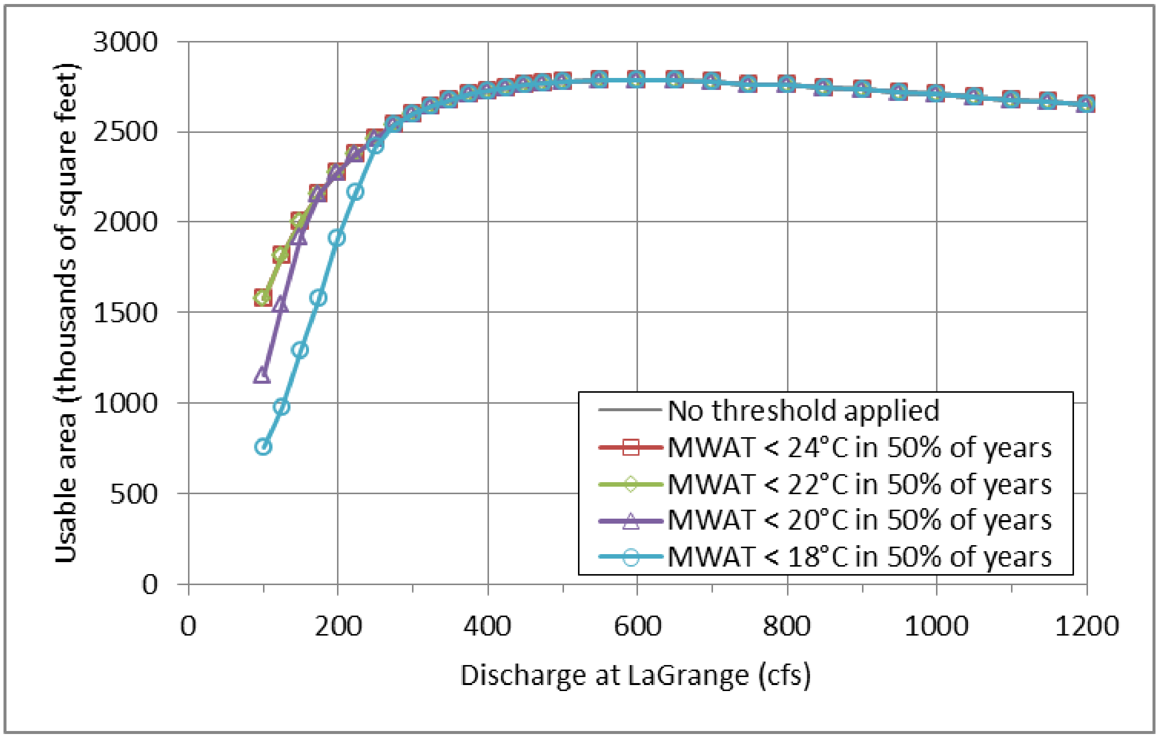

Figure C-39. Effective habitat for *O. mykiss* adult in July for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

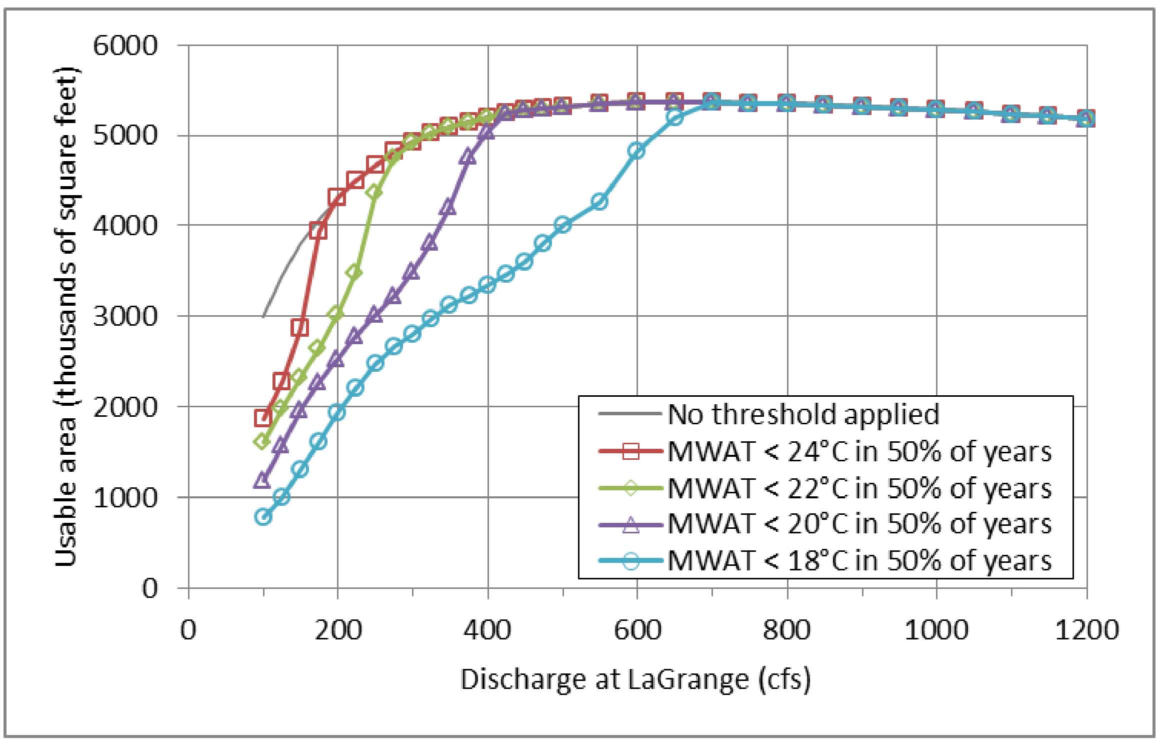

Figure C-40. Effective habitat for *O. mykiss* adult in July for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

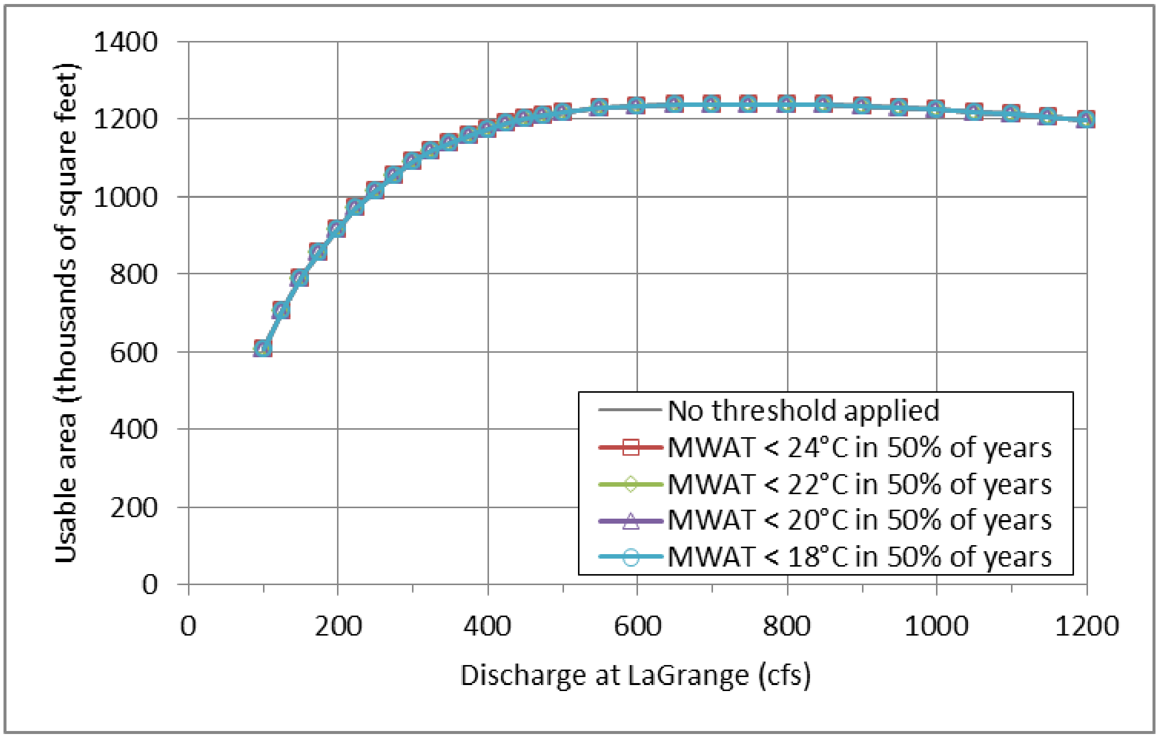

Figure C-41. Effective habitat for *O. mykiss* adult in August for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

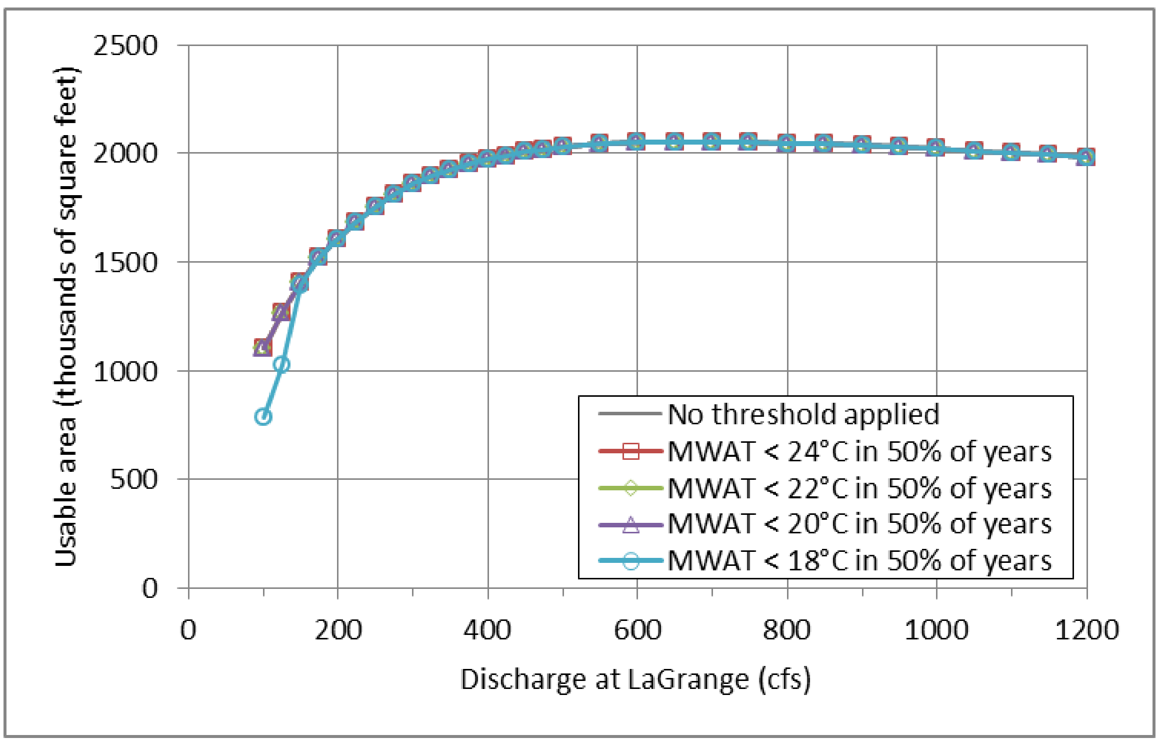

Figure C-42. Effective habitat for *O. mykiss* adult in August for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

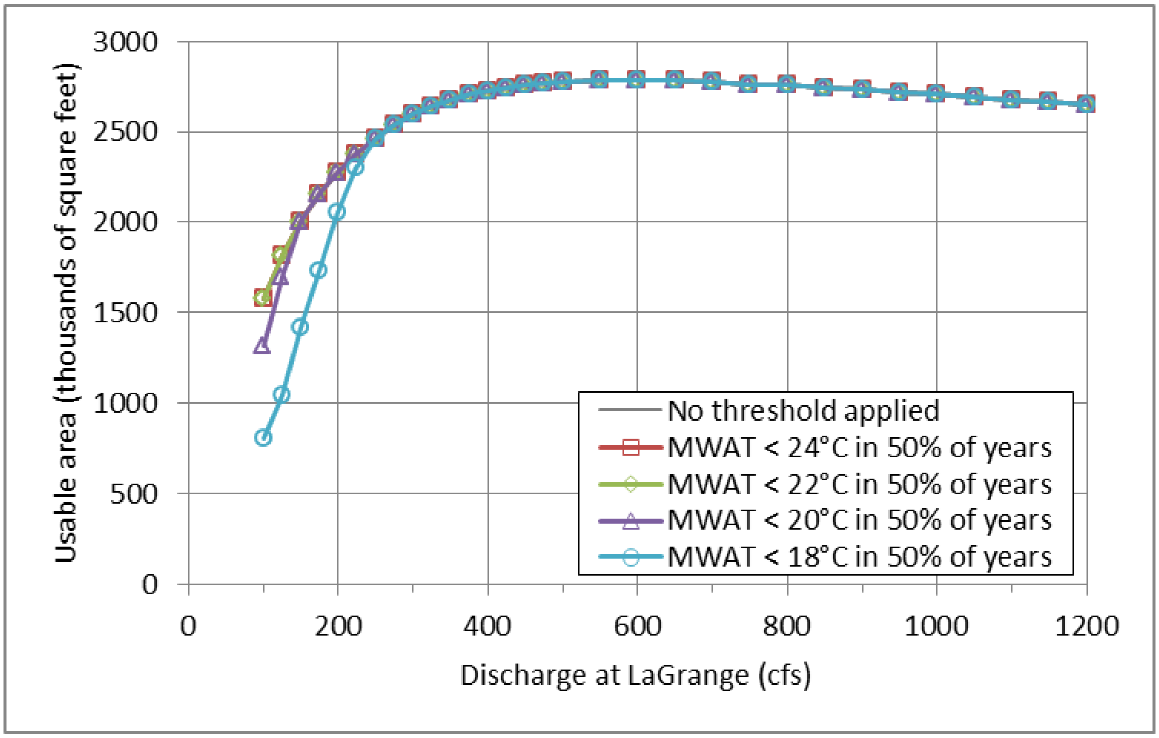

Figure C-43. Effective habitat for *O. mykiss* adult in August for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

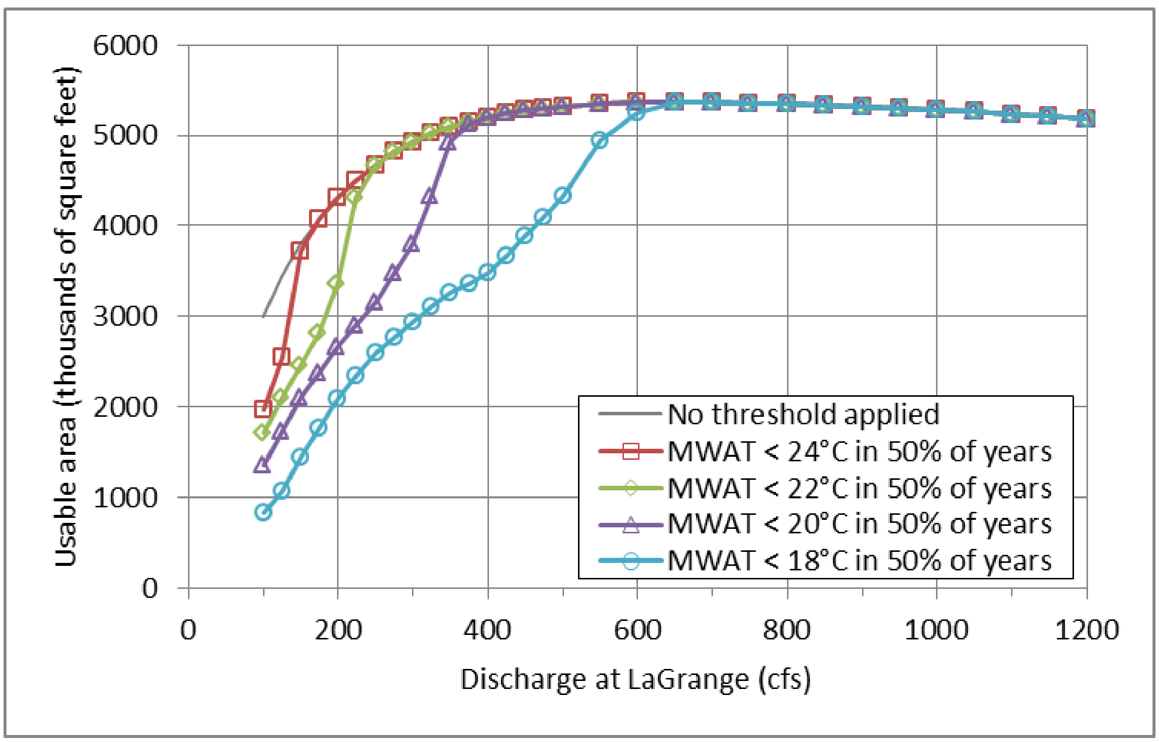

Figure C-44. Effective habitat for *O. mykiss* adult in August for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

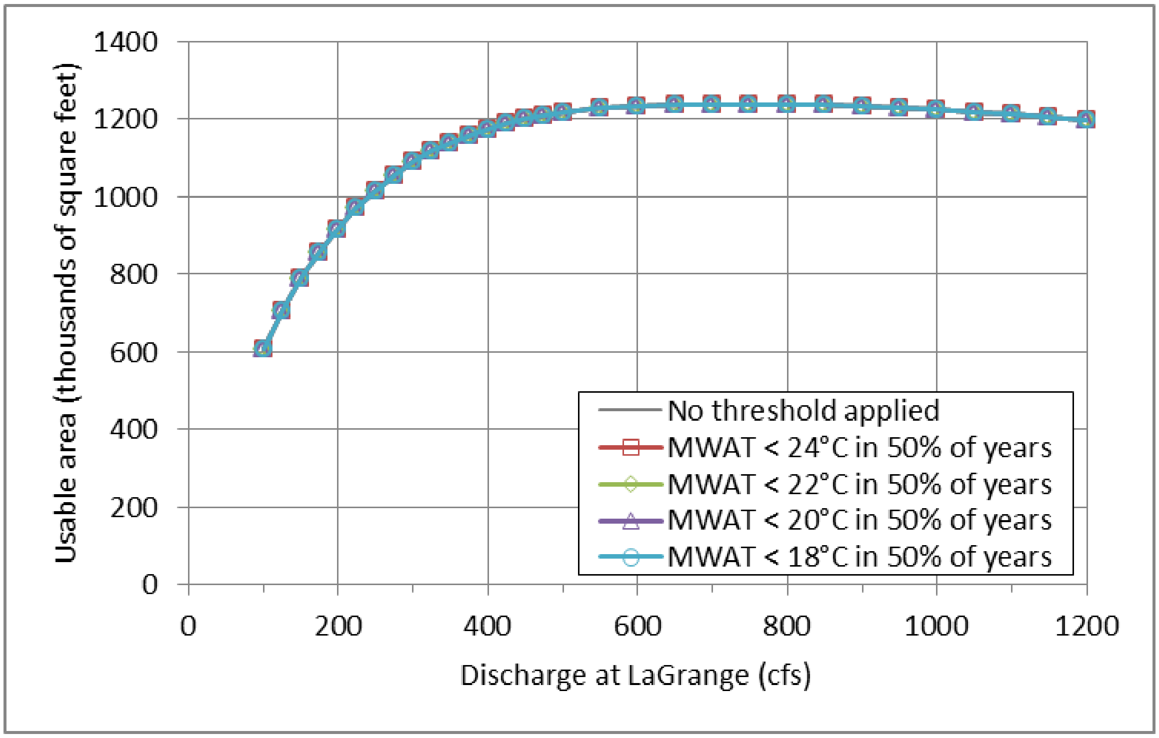

Figure C-45. Effective habitat for *O. mykiss* adult in September for habitats in sub-reach 1 (RM 51.9 to RM 46.9) meeting selected temperature thresholds.

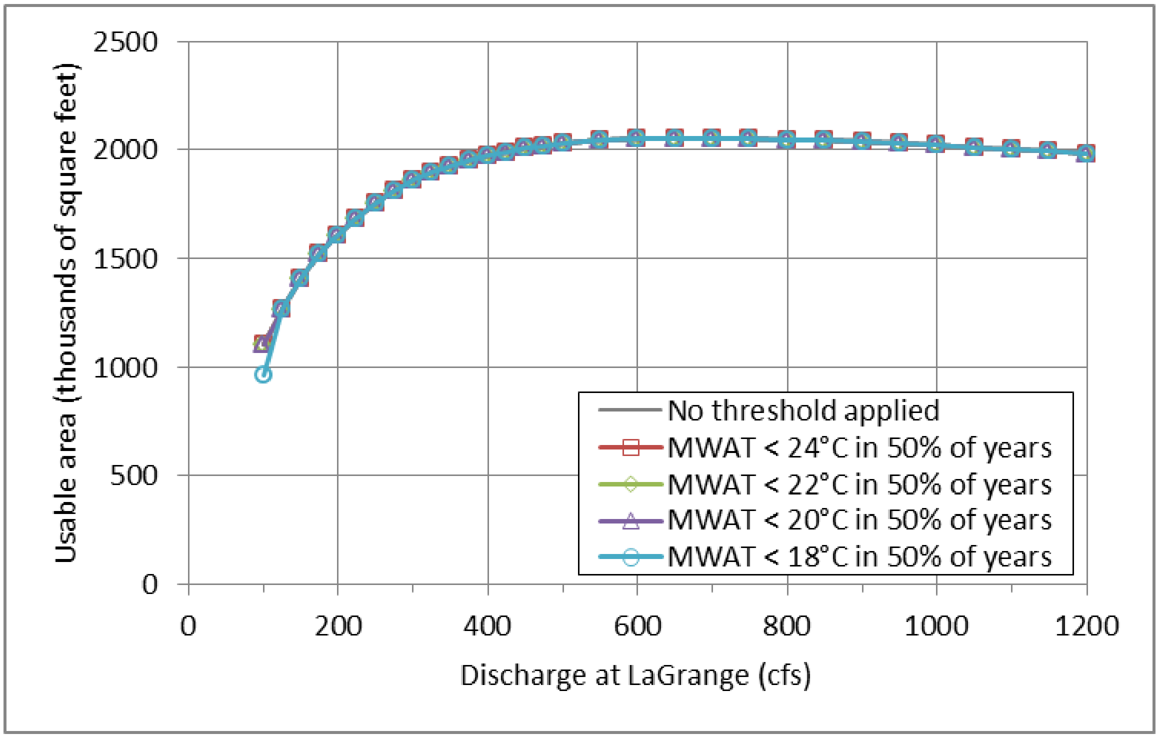

Figure C-46. Effective habitat for *O. mykiss* adult in September for habitats in sub-reaches 1-2 (RM 51.9 to RM 43.1) meeting selected temperature thresholds.

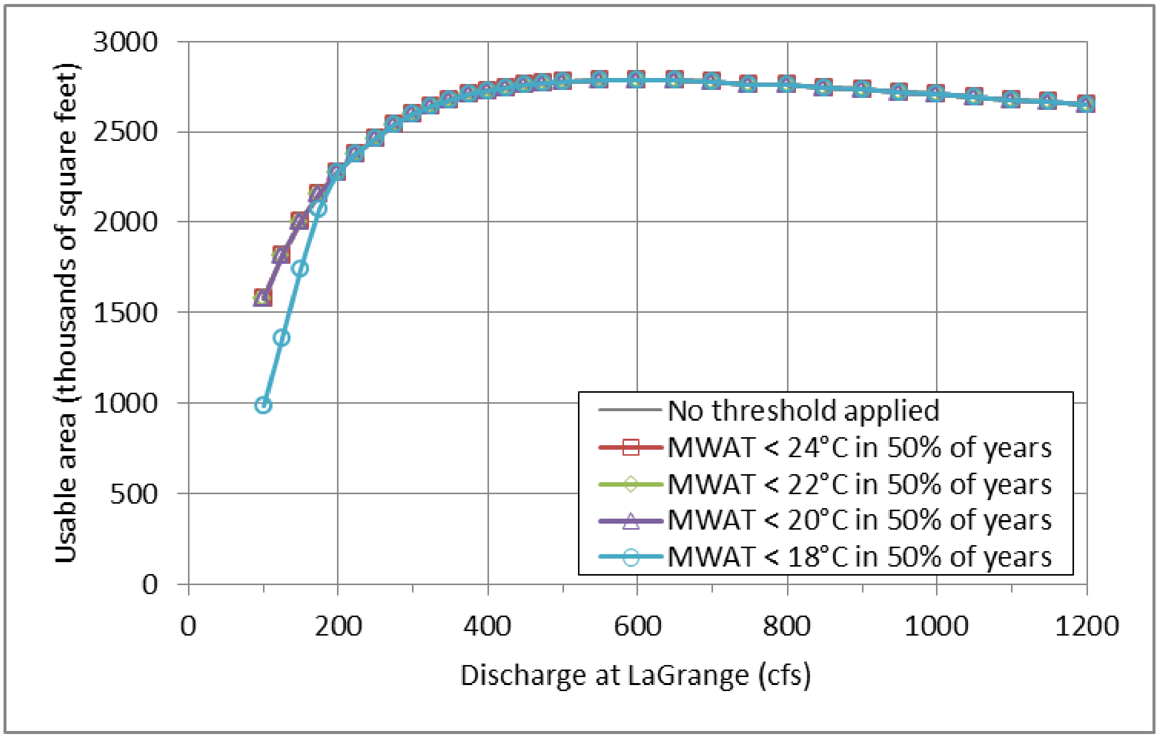

Figure C-47. Effective habitat for *O. mykiss* adult in September for habitats in sub-reaches 1-3 (RM 51.9 to RM 39.5) meeting selected temperature thresholds.

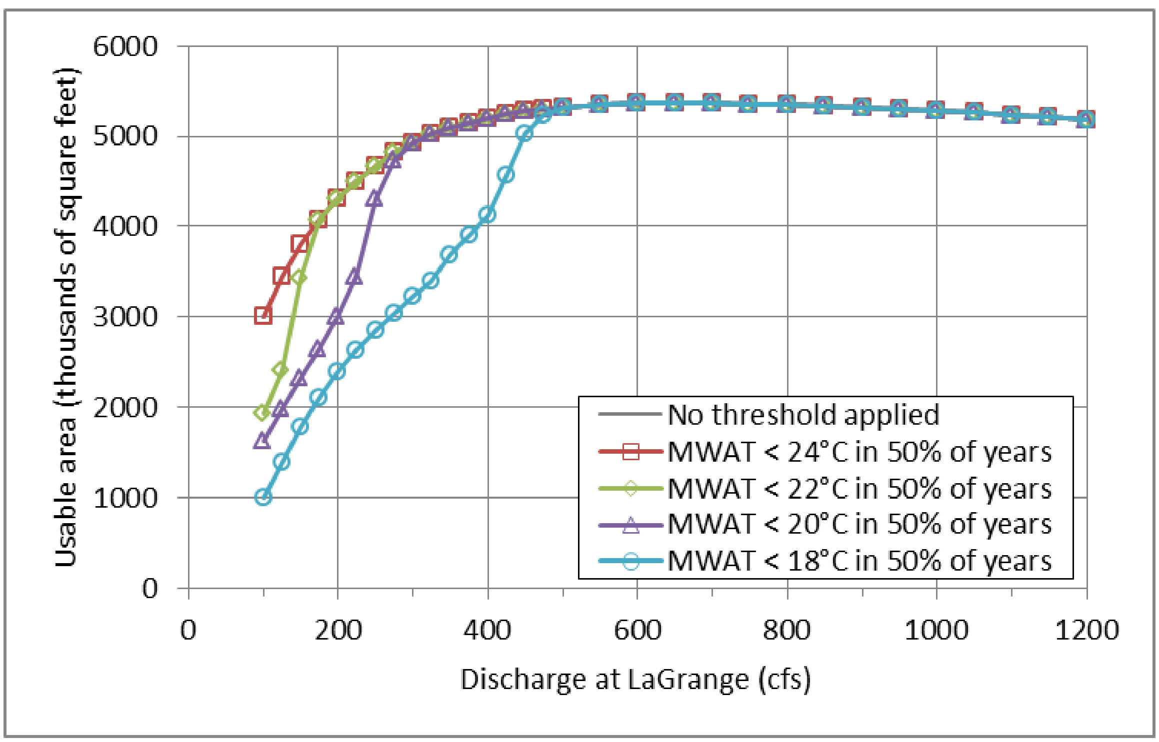

Figure C-48. Effective habitat for *O. mykiss* adult in September for habitats in sub-reaches 1-4 (RM 51.9 to RM 29.1) meeting selected temperature thresholds.

From: Chris Shutes
Sent: Monday, March 2, 2015 5:34 PM
To: Staples, Rose <Rose.Staples@hdrinc.com>
Cc: Ron Stork; Chandra Ferrari; John Buckley; Patrick Koepele; Peter Drekmeier; Julie Gantenbein ; Theresa Simsiman; Dave Steindorf; Amber Villalobos; Peter Barnes; Hughes, Robert; Tim Heyne; John Shelton; Murphey, Gretchen; Gordus, Andy; Alison Willy; Zac; Carl Mesick; Larry Thompson; John Wooster; Tom Holley; Steve Edmondson; Devine, John <John.Devine@hdrinc.com>; Brathwaite, Anna; Dias, Greg; Arthur Godwin; Noah Hume; Scott Wilcox
Subject: CSPA TRT comments on O. mykiss temp study

Dear Ms. Staples,

Attached please find the comments of California Sportfishing Protection Alliance and Tuolumne River Trust on the draft study entitled *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature.*

Please feel free to contact me if you have any questions.

Thank you.

Chris Shutes

Chris Shutes
FERC Projects Director
California Sportfishing Protection Alliance
510 421-2405

March 2, 2015

Ms. Rose Staples
HDR, Inc.
rose.staples@hdrinc.com

Re: Comments on January 31, 2015 draft of *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature.*

Dear Ms. Staples,

The California Sportfishing Protection Alliance (CSPA) and the Tuolumne River Trust (TRT) submit the following comments on the January 31, 2015 draft of *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature* ("Study").

Overview

Based on our review of the Study and some of the background material cited in the Study, including the EPA (2003) *Region 10 Guidance For Pacific Northwest State and Tribal Temperature Water Quality Standard* that the Study in significant part seeks to address, it appears to us that the Study proposes to recommend to regulators a change in the established EPA (2003) temperature benchmark for a 7DADM value for the population of *O. mykiss* in the lower Tuolumne River based on site-specific evidence.

The EPA (2003) guidelines recognize that site-specific thermal criteria for salmonids may be developed that are more appropriate for specific locations and populations than are the general criteria promulgated in the guidelines. Evaluation of physiological response in a target population is an appropriate approach to development of site-specific conditions. We accept the premise of the Study that site-specific physiological study of the response of fish to water temperature may demonstrate that such response in a specific population is different than broader, more general and geographically unspecific studies of the response of fish to water temperature have shown.

Neither CSPA nor the Tuolumne River Trust has fisheries physiologists on staff, and neither has the resources to hire a consulting fisheries physiologist at this time. We therefore have no comment at this time on the experimental approach adopted within the Study, the value of the metrics adopted, or the execution of the Study. We may bring in an outside consultant at a later point in the ILP process to evaluate these and other technical aspects of the Study.

Instead, we confine our comments to the implicit and explicit argument that Study results can "be used to determine a 7DADM value for this population." (Study Conclusion, p. 24).

The Study does not evaluate the physiological response of the population of *O. mykiss* in the lower Tuolumne River over time.

There are limitations to the Study that the Study does not acknowledge. Chief among these limitations is that the Study does not evaluate physiological response of the population of *O. mykiss* in the lower Tuolumne River over time. On the contrary, 75% of the test fish were sourced from a location one mile downstream of La Grange Powerhouse, where temperatures at capture ranged from 12.7° C to 17.1° C. While the Study is critical of Hokanson (1977) for an issue concerning confidence intervals, the Study does not address Hokanson's use of a 40-day period to evaluate physiological response. Other studies (e.g. Brett 1956; Bidgood 1969) similarly address long-term exposure to less-than-optimal thermal conditions. The Study does not acknowledge this limitation. It is akin to trying to determine the best overall athletic performance in a decathlon based on performance in the sprint alone.

Thermal conditions in the summer in most of the lower Tuolumne River are much more comparable to a marathon than a sprint. In the absence of adequate flow, grinding ambient temperatures with daily highs greater than 90° F for four months, and greater than 100° F on multiple days, create long-term water temperatures that are stressful to juvenile and adult *O. mykiss*. A City of San Francisco biologist has acknowledged on the record in this proceeding that *O. mykiss* populations in the lower Tuolumne River are substantially smaller than populations downstream of rim dams in the Sacramento River drainage, where water temperatures are generally much lower than temperatures in the lower Tuolumne River.[1] A change in the 7DADM value for the population of *O. mykiss* in the lower Tuolumne River is not warranted based on the evidence presented. The document should therefore be re-cast as a study, rather than walking what appears to us to be a gray line between a study and a position paper that advocates a departure from established guidance.

Before any adjustment to the established (EPA 2003) temperature benchmark for a 7DADM value for the population of *O. mykiss* in the lower Tuolumne River is considered based on site-specific conditions and response, further investigation and evaluation would be required. The Study should explicitly state this, and should describe additional evidence needed before any change in the 7DADM value for the population *O. mykiss* in the lower Tuolumne River might appropriately be evaluated.

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[1] *See* Dr. Ronald Yoshiyama, "*Commentary on Evaluating the Temperature-Related Flow Requirements of Steelhead-Rainbow Trout (Oncorhynchus Mykiss) in the Lower Tuolumne River: A Literature Review and Synthesis*," eLibrary no. 20120807-5082 (July 5, 2012), p. 2: "The actual numbers of adult and juvenile trout in the lower Tuolumne River were not accurately known until recently. Routine fish monitoring by the Districts indicates relatively low numbers of trout have been present over the past 1-2 decades--i.e., far below the numbers occurring in the Sacramento River mainstem and tributaries."

We discuss additional limitations of the Study and additional evidentiary needs below.

The Study results alone do not warrant site-specific summer water temperature criteria for *O. mykiss* in the lower Tuolumne River.

The Study is careful in its language not to state outright that its results *alone* can be used to develop alternative summer temperature criteria for the lower Tuolumne River. The Executive Summary states:

> Moreover, given that the average AAS remained within 5% of peak performance up to a temperature of 24.6°C and that all Tuolumne River *O. mykiss* maintained a FAS value >2.0 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, *be used to determine* a 7-Day Average of the Daily Maximum (7DADM) value. (Study, p. ii, emphasis added).

The Conclusion states in greater context:

> High quality field data were generated on the physiological performance of Tuolumne River *O. mykiss* acutely exposed to a temperature range of 13 to 25°C. These data on the RMR, MMR, AAS, and FAS were consistent with higher thermal performance in Tuolumne River *O. mykiss* compared to that used to generate the 7DADM value of 18°C using Pacific northwest *O. mykiss* (EPA 2003). These new data are consistent with recent peer-reviewed literature that points to local thermal adjustments among salmonid populations. Therefore, these data provide sound evidence to establish alternative numeric criteria that would apply to the Tuolumne River *O. mykiss* population below La Grange Diversion Dam. Given a measured Topt for AAS of 21.2°C, and that the average AAS remained within 5% of this peak performance up to 24.6°C, and all fish maintained a FAS value >2 up to 23°C, we recommend that a conservative upper performance limit of 22°C, instead of 18°C, *be used to determine* a 7DADM value for this population. (Study, p. 24, emphasis added)

The use of the passive voice ("be used to determine") is at once imprecise as to the nature and context of such use and imprecise as to who will or should use it. In our view, the appropriate use of the Study results would be to 1) evaluate their limitations; 2) develop additional investigations that might be necessary to scientifically justify consideration of adjusting thermal criteria for the population of *O. mykiss* in the lower Tuolumne River, 3) enumerate and evaluate regulatory and policy issues that might be involved in adjusting these criteria; and 4) assemble these necessary components and, based on this ensemble, develop a process for considering and evaluating site-specific water temperature criteria.

However, the Study provides no such context and proposes no such process. While the Study does not explicitly say that its results alone can be used to develop alternative summer temperature criteria for the lower Tuolumne River, the Districts have already used the results of the Study to advocate that temperatures greater than those of the EPA (2003) criteria be considered appropriate to determine amount of usable habitat in the lower Tuolumne. The draft

Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss distributed by the Districts' consultants to relicensing participants on February 27, 2015 adopts a higher range of suitable temperatures for over-summering O. mykiss *based on the present Thermal Performance Study*:

> Although the majority of historical (1996–2009) snorkel survey observations of *O. mykiss* in the lower Tuolumne River have occurred at temperatures of 20°C (68°F) or below (Ford and Kirihara 2010), *O. mykiss* have been routinely observed occupying Tuolumne River habitats at temperatures ranging from 11–25°C (52–77°C). Using wild juvenile *O. mykiss* collected from the Tuolumne River in the summer of 2014, a recently completed thermal performance study (Farrell et al. 2014) found a peak in the absolute aerobic scope (AAS) vs. temperature curve at 21.2°C (70°F), higher than the 19°C (66°F) growth rate optimum identified by Myrick and Cech (2001). Because Farrell et al. (2014) also found that the AAS of the wild *O. mykiss* test fish remained within 5% of the peak AAS between 17.8°C (64°F) to 24.6°C (76°F), these site-specific empirical data with broader temperature thresholds were selected for evaluation of thermal suitability for *O. mykiss*. In the current study, the temperatures of 18°C (66.4°F), 20°C (68°F), 22°C (71.6°F), and 24°C (75.2°F) were evaluated over each of the summer months (June through September) when these temperatures can be exceeded in the lower Tuolumne River.[2]

In skipping from study to study, any caveats and limitations that might be present or implied disappear. In order to avoid such misuse, the authors of the current Study should be more explicit in its caveats and should describe the limitations of its conclusions.

The Study may be limited because it analyzes a single lifestage.

The Study examines only the juvenile lifestage of *O. mykiss* in the lower Tuolumne River. The Clean Water Act requires that the most sensitive resources be protected. It is not clear whether the adult lifestage, which is also present during the summer time period, is more, equally or less sensitive to high water temperatures. Before adjustments of summer temperature criteria for *O. mykiss* in the lower Tuolumne River could be considered, an evaluation of the physiological response of adult *O. mykiss* in the lower Tuolumne River would need to conducted, in addition to completing the evaluation of the physiological response of juveniles.

The Study makes comparisons between *O. mykiss* in the lower Tuolumne River and populations that are more permanent and defined and that have more common characteristics.

The Study draws comparisons with other populations of rainbow trout that have demonstrated higher temperature tolerances than the figures given for juvenile rearing in the EPA (2003) Criteria. Several of these are cited in the EPA document, including redband trout in Eastern Oregon, southern California coastal steelhead, and trout introduced in Australia.

[2] Stillwater Sciences, 2015, *Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss*. Draft Report. Prepared by Stillwater Sciences, Davis, California for Turlock Irrigation District, Turlock California and Modesto Irrigation District, Modesto, California. Distributed to relicensing participants via e-mail by Ms. Rose Staples on February 27, 2015, pp. 2-3.

Certainly at least the redband and southern California steelhead are more likely to share common ancestry and even genetics than the fish in the lower Tuolumne River, where the population was extremely small due to low project flows until 1995. The current Tuolumne population is likely a combination of residual lower river fish, wild or hatchery fish washed down from La Grange (themselves possibly the result of production in La Grange Reservoir or originating in Don Pedro Reservoir), and some number of anadromous individuals of unknown origin and their progeny. It is further likely that the population is being replenished from these sources on an ongoing basis, and that some portion of the fish that are there in several years will have little directly in common with the current population. This is particularly likely under dry or drought conditions, when a greater proportion of the existing population may be expected to perish. Managing a changing population based on ascribed thermal tolerances of an existing population is questionable both scientifically and as policy.

It is likely that the present population in the lower Tuolumne is temperature tolerant because it has had to be in order to survive, and that improved thermal conditions would create a larger population. Improved thermal conditions would certainly increase the volume of suitable habitat by pushing thermal limitations further downstream. It is a policy as well as a scientific question whether to manage to the highest suitable temperature (whatever that may be) or to manage to what is likely to produce a stronger population. On a policy and recreational basis, it is hard to justify a small population managed for small fish. If the population were more robust, the argument for managing to a higher temperature would be more credible.

There is no bioenergetics study of O. mykiss in the lower Tuolumne River that would support management for water temperatures higher than those recommended in EPA (2003) guidance.

The Districts declined in 2011 to conduct a bioenergetics study of O. mykiss in the lower Tuolumne River as recommended by the Department of Fish and Wildlife.[3] The Commission did not order this study. The current Study recognizes: "the important ecological consideration is whether or not there is sufficient food in the Tuolumne River to support the highest MR associated with high temperature." (Study, p. 22). The Study supports the hypothesis that sufficient food is present only with anecdotal data:

> All available studies suggest that the Tuolumne River population is not food limited, including direct studies of Tuolumne River Chinook salmon diet (TID/MID 1992, Appendix 16), long-term benthic macro-invertebrate sampling data collected from 1988–2008 (e.g., TID/MID 1997, Report 1996-4; TID/MID 2009, Report 2008-7), as well as the relatively high length-at-age for *O. mykiss* sampled in 2012 (Stillwater Sciences 2013). Indeed, the *O. mykiss* sampled for the current study were apparently feeding well in the river during summer months given the high condition factors (see Appendix 2), feces being regularly found in the swim tunnel and two test fish regurgitating rather large meals post-exhaustion. (*ibid*).

[3] *See* California Department of Fish and Wildlife, *Comments on Proposed Study Plan*, eLibrary 20111024-5118, p. 55 ff., proposed Bioenergetics Study.

It is one thing to say that there is apparently sufficient food in the lower Tuolumne for the small population of *O. mykiss* located in a relatively small section of the river. It is quite another to argue in the absence of a targeted study that food production is great enough to support a larger population at the highest metabolic rate associated with high water temperatures. There is no evidence to support such a finding. If food is indeed unusually abundant, why is the *O. mykiss* population in the lower Tuolumne River neither greatly abundant nor characterized by large numbers of large fish?

Conclusion and recommendations

The summer water temperature criteria that are apparently recommended in the Study, and that are more definitively recommended based on the present Study in the just-released draft study entitled *Lower Tuolumne River Instream Flow Study—Evaluation of effective usable habitat area for over-summering O. mykiss*, are not warranted by the evidence the Study has collected. If the Districts wish to persist in seeking to define site-specific summer water temperature criteria for the lower Tuolumne River, they should affirmatively address the scientific and policy issues we have described above. In brief, these are

1. Follow-up site specific physiological studies must address elevated water temperatures over an extended period of time, ideally over an entire summer.
2. Follow-up site specific physiological studies must be conducted on adult as well as juvenile *O. mykiss*.
3. Follow-up site specific physiological studies must address the likely multiple sources and ongoing replenishment of the *O. mykiss* population of the lower Tuolumne River.
4. The Districts should perform a bioenergetics study for juvenile and adult *O. mykiss* in the lower Tuolumne River.

In addition, the Study should be edited so that the Executive Summary and the Conclusion place the value of the findings in the appropriate context of how they might inform a comprehensive review of site-specific summer thermal conditions in the lower Tuolumne River.

Please contact Chris Shutes if you have any questions. Thank you for the opportunity to comment on the draft of the Study entitled *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature*.

Respectfully submitted,

Patrick Koepele

Chris Shutes

Patrick Koepele
Executive Director
Tuolumne River Trust
patrick@tuolumne.org

Chris Shutes
FERC Projects Director
California Sportfishing Protection Alliance
blancapaloma@msn.com

From: Staples, Rose
Sent: Monday, March 16, 2015 11:40 AM
To: 'Barnes, Peter@Waterboards' <Peter.Barnes@waterboards.ca.gov>
Subject: RE: Don Pedro Study Report for your Review and Comment

Thank you for the advisory; I will let the Districts know.

Rose Staples, CAP-OM
D 207-239-3857

hdrinc.com/follow-us

From: Barnes, Peter@Waterboards [mailto:Peter.Barnes@waterboards.ca.gov]
Sent: Monday, March 16, 2015 11:39 AM
To: Staples, Rose
Subject: RE: Don Pedro Study Report for your Review and Comment

Rose,

I will be unable to submit my comments today. They have not completed review. We have had some other things come up (on going drought, etc.) which have required management's attention. I will have them completed and to you by the COB Wednesday at the latest. I apologize for the delay.

Peter

From: Staples, Rose [Rose.Staples@hdrinc.com]
Sent: Wednesday, March 04, 2015 10:31 AM
To: Barnes, Peter@Waterboards
Subject: RE: Don Pedro Study Report for your Review and Comment

My apologies for taking so long to get back to you on this question. I am advised that it will be okay for you to submit your comments on this Study Report by the 16th of March. Thank you.

Rose Staples, CAP-OM
D 207-239-3857

hdrinc.com/follow-us

From: Staples, Rose
Sent: Monday, March 02, 2015 3:26 PM
To: 'Barnes, Peter@Waterboards'
Subject: RE: Don Pedro Study Report for your Review and Comment

I have forwarded this question to the Districts; will get back to you shortly.

Rose Staples, CAP-OM
D 207-239-3857

hdrinc.com/follow-us

From: Barnes, Peter@Waterboards [mailto:Peter.Barnes@waterboards.ca.gov]
Sent: Monday, March 02, 2015 2:47 PM
To: Staples, Rose
Subject: RE: Don Pedro Study Report for your Review and Comment

Rose,

In order to supply detailed comments on this study report, I am requesting an extension of two weeks. Due to project workload, I have not been able to give this report the attention it needs. Please let me know if I can have an extension until March 16, 2015 to submit my comments. Thank you.

Peter Barnes

From: Staples, Rose [mailto:Rose.Staples@hdrinc.com]
Sent: Friday, January 30, 2015 10:20 AM
Subject: Don Pedro Study Report for your Review and Comment

Please find attached for your review and comment a study report entitled *Thermal Performance of Wild Juvenile Oncorhynchus Mykiss in the Lower Tuolumne River: A Case for Local Adjustment to High River Temperature.*

This study was conducted as part of W&AR-14: Assessment of Temperature Criteria for the Don Pedro Hydroelectric Project by a team of UC Davis scientists under the direction of Dr. Nann Fangue and Dr. Anthony Farrell (University of British Columbia). The researchers investigated the thermal performance of juvenile Tuolumne River *O. mykiss* with respect to the seasonal maxima water temperatures the fish experience during the summer months. The UC Davis Team and Dr. Farrell tested wild locally caught *O. mykiss* in a swim tunnel respirometer as described in the report.

Please provide any comments you may have by March 2, 2015 to me at rose.staples@hdrinc.com.

Thank you.

Rose Staples, CAP-OM
Executive Assistant

HDR
970 Baxter Boulevard Suite 301
Portland ME 04103
D 207-239-3857
rose.staples@hdrinc.com

hdrinc.com/follow-us

From: Staples, Rose
Sent: Thursday, March 12, 2015 7:53 PM
Cc: Staples, Rose <Rose.Staples@hdrinc.com>
Subject: Districts E-File with FERC Request for Extension of Time to Conduct Predation Study

The Districts e-filed with FERC today a request for an additional one-year extension of the FERC-approved 2014 Predation Study Plan. This extension would extend completion of field work into 2016, with the study report to be filed in April 2017. A copy of the filing is attached to today's ANNOUNCEMENT on the Don Pedro Relicensing Website at www.donpedro-relicensing.com and is also accessible via FERC's E-Library at www.ferc.gov. If you have any difficulties locating and /or downloading this document, please let me know. Thank you.

Rose Staples, CAP-OM
Executive Assistant

HDR
970 Baxter Boulevard Suite 301
Portland ME 04103
D 207-239-3857
rose.staples@hdrinc.com

hdrinc.com/follow-us

From: Staples, Rose
BCC To: Don Pedro Relicensing Participants Email Group
Sent: Monday, March 16, 2015 4:50 PM
Cc: Staples, Rose <Rose.Staples@hdrinc.com>
Subject: Don Pedro W-AR-11 Study Report for Review and Comment

To Don Pedro Relicensing Participants:

Please find attached for your review and comment the W&AR-11 ***Chinook Salmon Otolith Study Report***.

The study's objectives were to use otolith microstructural growth patterns and/or microchemistry in order to identify whether returning adults originated from hatcheries or riverine environments other than the Tuolumne River and to identify growth rates and sizes of 'wild' fish at exit from the Tuolumne River and from the freshwater Delta.

Please provide any comments to me at rose.staples@hdrinc.com by April 15, 2015.

Thank you.

Rose Staples, CAP-OM
Executive Assistant

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970 Baxter Boulevard Suite 301
Portland ME 04103
D 207-239-3857
rose.staples@hdrinc.com

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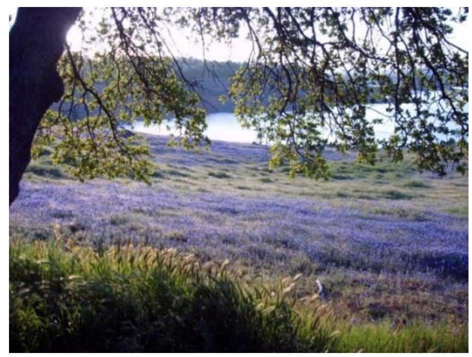
CHINOOK SALMON OTOLITH STUDY

DRAFT REPORT – CONFIDENTIAL

(NOT FOR CITATION)

DON PEDRO PROJECT

FERC NO. 2299



WATER & POWER

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Modesto Irrigation District

Prepared for:

Turlock Irrigation District – Turlock, California

Modesto Irrigation District – Modesto, California

Prepared by:

Stillwater Sciences

March 2015

This study has involved the cooperation and participation of the
California Department of Fish and Wildlife
and Dr. Rachel Johnson and Dr. Anna Sturrock
at the University of California Davis,
Department of Animal Science.

Chinook Salmon Otolith Study
Study Report

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List of Acronyms

ac ....acres

ACEC..........................Area of Critical Environmental Concern

AF ....acre-feet

ACOE..........................U.S. Army Corps of Engineers

AFY............................acre-feet per year

ADA............................Americans with Disabilities Act

ALJ.............................Administrative Law Judge

APE............................Area of Potential Effect

ARMR........................Archaeological Resource Management Report

BA..............................Biological Assessment

BAWSCA ....Bay Area Water Supply Conservation Agency

BDCP.........................Bay-Delta Conservation Plan

BEA............................Bureau of Economic Analysis

BLM...........................U.S. Department of the Interior, Bureau of Land Management

BLM-S ....Bureau of Land Management – Sensitive Species

BMI............................Benthic macroinvertebrates

BMP...........................Best Management Practices

BO..............................Biological Opinion

CAISO........................California Independent System Operators

CalEPPC ....California Exotic Pest Plant Council

CalSPA.......................California Sports Fisherman Association

CALVIN ....California Value Integrated Network

CAS............................California Academy of Sciences

CASFMRA ....California Chapter of the American Society of Farm Managers and Rural Appraisers

CCC............................Criterion Continuous Concentrations

CCIC..........................Central California Information Center

CCSF..........................City and County of San Francisco

CCVHJV....................California Central Valley Habitat Joint Venture

CD..............................Compact Disc

CDBW........................California Department of Boating and Waterways

CDEC........................California Data Exchange Center
CDFA........................California Department of Food and Agriculture
CDFG........................California Department of Fish and Game (as of January 2013, Department of Fish and Wildlife)
CDMG.......................California Division of Mines and Geology
CDOF........................California Department of Finance
CDP...........................Census Designated Place
CDPH........................California Department of Public Health
CDPR........................California Department of Parks and Recreation
CDSOD.....................California Division of Safety of Dams
CDWR.......................California Department of Water Resources
CE ....California Endangered Species
CEII...........................Critical Energy Infrastructure Information
CEQA........................California Environmental Quality Act
CESA ....California Endangered Species Act
CFR...........................Code of Federal Regulations
cfs..............................cubic feet per second
CGS...........................California Geological Survey
CMAP ....California Monitoring and Assessment Program
CMC..........................Criterion Maximum Concentrations
CNDDB.....................California Natural Diversity Database
CNPS.........................California Native Plant Society
CORP ....California Outdoor Recreation Plan
CPI ....Consumer Price Index
CPUE ....Catch Per Unit Effort
CRAM.......................California Rapid Assessment Method
CRLF.........................California Red-Legged Frog
CRRF ....California Rivers Restoration Fund
CSAS.........................Central Sierra Audubon Society
CSBP.........................California Stream Bioassessment Procedure
CT ....Census Tract
CT ....California Threatened Species
CTR...........................California Toxics Rule

CTS ....California Tiger Salamander
CUWA ....California Urban Water Agency
CV ....Contingent Valuation
CVP............................Central Valley Project
CVPIA........................Central Valley Project Improvement Act
CVRWQCB ....Central Valley Regional Water Quality Control Board
CWA ....Clean Water Act
CWD ....Chowchilla Water District
CWHR........................California Wildlife Habitat Relationship
CWT...........................hundredweight
Districts......................Turlock Irrigation District and Modesto Irrigation District
DLA ....Draft License Application
DPRA.........................Don Pedro Recreation Agency
DO..............................Dissolved Oxygen
DPS ....Distinct Population Segment
EA ....Environmental Assessment
EC ....Electrical Conductivity
EDD ....Employment Development Department
EFH............................Essential Fish Habitat
EIR ....Environmental Impact Report
EIS..............................Environmental Impact Statement
ENSO ....El Nino – Southern Oscillation
EO ....Executive Order
EPA............................U.S. Environmental Protection Agency
ERS ....Economic Research Service (USDA)
ESA............................Federal Endangered Species Act
ESRCD.......................East Stanislaus Resource Conservation District
ESU ....Evolutionary Significant Unit
ET...............................Evapotranspiration
EVC............................Existing Visual Condition
EWUA........................Effective Weighted Useable Area
FEMA ....Federal Emergency Management Agency
FERC..........................Federal Energy Regulatory Commission

FFS............................Foothills Fault System
FL...............................Fork length
FMU...........................Fire Management Unit
FMV...........................Fair Market Value
FOT............................Friends of the Tuolumne
FPC ....Federal Power Commission
FPPA..........................Federal Plant Protection Act
FPC ....Federal Power Commission
ft.................................feet
ft/mi............................feet per mile
FWCA........................Fish and Wildlife Coordination Act
FYLF..........................Foothill Yellow-Legged Frog
g..................................grams
GAMS........................General Algebraic Modeling System
GIS ....Geographic Information System
GLO ....General Land Office
GPM...........................Gallons per Minute
GPS ....Global Positioning System
HCP............................Habitat Conservation Plan
HHWP........................Hetch Hetchy Water and Power
HORB ....Head of Old River Barrier
HPMP.........................Historic Properties Management Plan
ILP..............................Integrated Licensing Process
IMPLAN ....Impact analysis for planning
I-O..............................Input-Output
ISR ....Initial Study Report
ITA.............................Indian Trust Assets
kV...............................kilovolt
LTAM ....Long-Term Acoustic Monitoring
LTR............................Lower Tuolumne River
m ....meters
M&I............................Municipal and Industrial
MCL...........................Maximum Contaminant Level

mg/kg ....milligrams/kilogram
mg/L..........................milligrams per liter
mgd ....million gallons per day
mi ....miles
mi^2 ....square miles
MID...........................Modesto Irrigation District
MOU ....Memorandum of Understanding
MRP ....Monitoring and Reporting Program
MRWTP.....................Modesto Regional Water Treatment Plant
MSCS.........................Multi-Species Conservation Strategy
msl.............................mean sea level
MVA ....Megavolt Ampere
MW ....megawatt
MWh ....megawatt hour
mya............................million years ago
NAE ....National Academy of Engineering
NAHC ....Native American Heritage Commission
NAICS........................North America Industrial Classification System
NAS...........................National Academy of Sciences
NASS ....National Agricultural Statistics Service (USDA)
NAVD 88...................North American Vertical Datum of 1988
NAWQA ....National Water Quality Assessment
NCCP ....Natural Community Conservation Plan
NEPA ....National Environmental Policy Act
ng/g ....nanograms per gram
NGOs ....Non-Governmental Organizations
NHI ....Natural Heritage Institute
NHPA.........................National Historic Preservation Act
NISC ....National Invasive Species Council
NMFS.........................National Marine Fisheries Service
NMP...........................Nutrient Management Plan
NOAA........................National Oceanic and Atmospheric Administration
NOI ....Notice of Intent

NPS ............................U.S. Department of the Interior, National Park Service
NRCS ....National Resource Conservation Service
NRHP..........................National Register of Historic Places
NRI.............................Nationwide Rivers Inventory
NTU ....Nephelometric Turbidity Unit
NWI............................National Wetland Inventory
NWIS ....National Water Information System
NWR ....National Wildlife Refuge
NGVD 29 ....National Geodetic Vertical Datum of 1929
O&M ....operation and maintenance
OEHHA......................Office of Environmental Health Hazard Assessment
OID ....Oakdale Irrigation District
ORV ....Outstanding Remarkable Value
PAD............................Pre-Application Document
PDO............................Pacific Decadal Oscillation
PEIR ....Program Environmental Impact Report
PGA............................Peak Ground Acceleration
PHG............................Public Health Goal
PM&E ....Protection, Mitigation and Enhancement
PMF............................Probable Maximum Flood
PMP............................Positive Mathematical Programming
POAOR ....Public Opinions and Attitudes in Outdoor Recreation
ppb..............................parts per billion
ppm ....parts per million
PSP ....Proposed Study Plan
QA ....Quality Assurance
QC ....Quality Control
RA ....Recreation Area
RBP ....Rapid Bioassessment Protocol
Reclamation ...............U.S. Department of the Interior, Bureau of Reclamation
RM ....River Mile
RMP ....Resource Management Plan
RP...............................Relicensing Participant

RR ....Recreation Resources
RSP ....Revised Study Plan
RST ....Rotary Screw Trap
RWF...........................Resource-Specific Work Groups
RWG ....Resource Work Group
RWQCB.....................Regional Water Quality Control Board
SC...............................State candidate for listing under CESA
SCD............................State candidate for delisting under CESA
SCE ....State candidate for listing as endangered under CESA
SCT ....State candidate for listing as threatened under CESA
SD1 ....Scoping Document 1
SD2 ....Scoping Document 2
SE...............................State Endangered Species under the CESA
SFP.............................State Fully Protected Species under CESA
SFPUC ....San Francisco Public Utilities Commission
SHPO ....State Historic Preservation Office
SIC ....Standard Industry Classification
SJR.............................San Joaquin River
SJRA ....San Joaquin River Agreement
SJRGA ....San Joaquin River Group Authority
SJTA ....San Joaquin River Tributaries Authority
SPD ....Study Plan Determination
SRA............................State Recreation Area
SRMA ....Special Recreation Management Area or Sierra Resource Management Area (as per use)
SRMP.........................Sierra Resource Management Plan
SRP ....Special Run Pools
SSC ....State species of special concern
ST...............................California Threatened Species under the CESA
STORET ....Storage and Retrieval
SWAMP.....................Surface Water Ambient Monitoring Program
SWAP ....Statewide Agricultural Model
SWE...........................Snow-Water Equivalent

SWP ....State Water Project
SWRCB......................State Water Resources Control Board
TAC............................Technical Advisory Committee
TAF ....thousand acre-feet
TC ....Travel Cost
TCP ....Traditional Cultural Properties
TDS ....Total Dissolved Solids
TID ....Turlock Irrigation District
TIN .... Triangular Irregular Network
TMDL ....Total Maximum Daily Load
TOC............................Total Organic Carbon
TPH ....Total Petroleum hydrocarbon
TRT ....Tuolumne River Trust
TRTAC ....Tuolumne River Technical Advisory Committee
UC ....University of California
UCCE.........................University of California Cooperative Extension
USDA.........................U.S. Department of Agriculture
USDOC......................U.S. Department of Commerce
USDOI .......................U.S. Department of the Interior
USFS ..........................U.S. Department of Agriculture, Forest Service
USFWS ......................U.S. Department of the Interior, Fish and Wildlife Service
USGS .........................U.S. Department of the Interior, Geological Survey
USR............................Updated Study Report
UTM...........................Universal Transverse Mercator
VAMP ....Vernalis Adaptive Management Plan
VELB ....Valley Elderberry Longhorn Beetle
VES ....Visual Encounter Surveys
VRM ....Visual Resource Management
W&AR ....Water & Aquatic Resources
WMP ....Waste Management Plan
WPT ....Western Pond Turtle
WSA...........................Wilderness Study Area
WSIP ....Water System Improvement Program

WTP ....Willingness to Pay

WWTP ....Wastewater Treatment Plant

WY ....water year

μS/cm ....microSiemens per centimeter

1.0 INTRODUCTION

1.1 Background

Turlock Irrigation District (TID) and Modesto Irrigation District (MID) (collectively, the Districts) are the co-licensees of the 168-megawatt (MW) Don Pedro Project (Project) located on the Tuolumne River in western Tuolumne County in the Central Valley region of California. The Don Pedro Dam is located at river mile (RM) 54.8 and the Don Pedro Reservoir has a normal maximum water surface elevation of 830 ft above mean sea level (msl; NGVD 29). At elevation 830 ft, the reservoir stores over 2,000,000 acre-feet (AF) of water and has a surface area slightly less than 13,000 acres (ac). The watershed above Don Pedro Dam is approximately 1,533 square miles (mi^2). The Project is designated by the Federal Energy Regulatory Commission (FERC) as project no. 2299.

Both TID and MID are local public agencies authorized under the laws of the State of California to provide water supply for irrigation and municipal and industrial (M&I) uses and to provide retail electric service. The Don Pedro Project serves many purposes including providing water storage for the beneficial use of irrigation of over 200,000 ac of prime Central Valley farmland and for the use of M&I customers in the City of Modesto (population 210,000). Consistent with agreements between the Districts and City and County of San Francisco (CCSF), the Don Pedro Reservoir also includes a “water bank” of up to 570,000 AF of storage which CCSF uses to efficiently manage the water supply from its Hetch Hetchy water system while meeting the senior water rights of the Districts. The “water bank” within Don Pedro Reservoir provides significant benefits for CCSF’s 2.6 million customers in the San Francisco Bay Area.

The Don Pedro Project also provides storage for flood management purposes in the Tuolumne and San Joaquin rivers in coordination with the U.S. Army Corps of Engineers (ACOE). Other important uses supported by the Don Pedro Project are recreation, protection of aquatic resources in the lower Tuolumne River, and hydropower generation.

The Project Boundary extends from RM 53.2, which is one mile below the Don Pedro powerhouse, upstream to RM 80.8 at a water surface elevation of 845 ft (31 FPC ¶ 510 [1964]). The Project Boundary encompasses approximately 18,370 ac with 74 percent of the lands owned jointly by the Districts and the remaining 26 percent (approximately 4,802 ac) owned by the United States and managed as a part of the U.S. Bureau of Land Management (BLM) Sierra Resource Management Area.

The primary Don Pedro Project facilities include the 580-foot-high Don Pedro Dam and Reservoir completed in 1971; a four-unit powerhouse situated at the base of the dam; related facilities including the Project spillway, outlet works, and switchyard; four dikes (Gasburg Creek Dike and Dikes A, B, and C); and three developed recreational facilities (Fleming Meadows, Blue Oaks, and Moccasin Point Recreation Areas). The location of the Don Pedro Project and its primary facilities is shown in Figure 1.1-1.

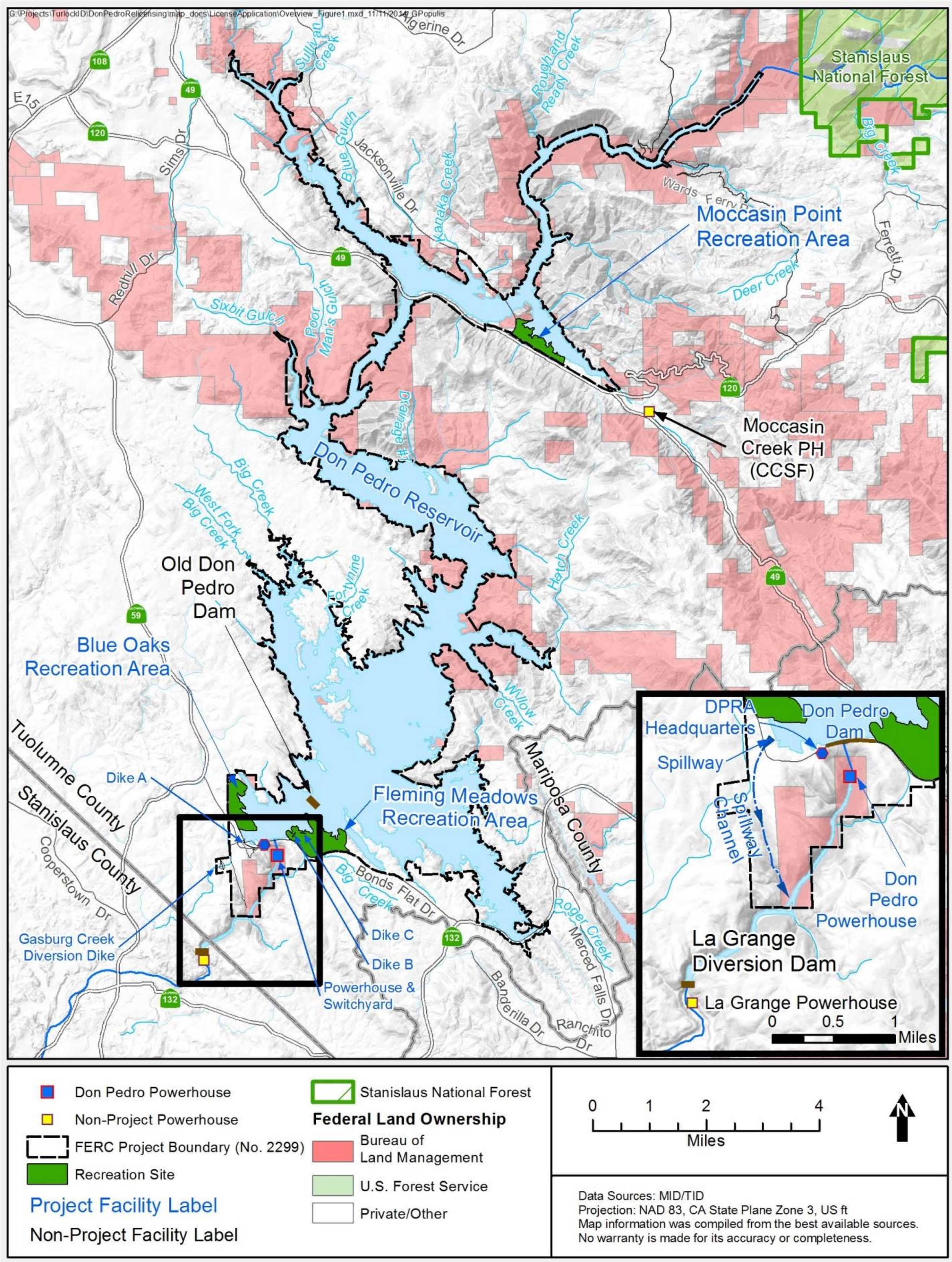

Figure 1.1-1. Don Pedro Project site location map.

1.2 Relicensing Process

The current FERC license for the Project expires on April 30, 2016, and the Districts applied for a new license on April 30, 2014. At that time, and consistent with study schedules approved by FERC through the Integrated Licensing Process (ILP) study plan determinations, five important studies involving the resources of the lower Tuolumne River were still in-progress. These studies are scheduled to be completed by April 2016. Once these studies are completed, the Districts will evaluate all data, reports, and models then available for the purpose of identifying appropriate protection, mitigation, and enhancement (PM&E) measures to address the direct, indirect, and cumulative effects of Project operations and maintenance. Upon completion of this evaluation, the Districts will prepare any needed amendments to the license application. The Districts have projected November 2016 as the date for filing any required amendments to the license application.

The Districts began the relicensing process by filing a Notice of Intent and Pre-Application Document (PAD) with FERC on February 10, 2011, in accordance with the regulations governing the ILP. The Districts' PAD included descriptions of the Project facilities, operations, license requirements, and Project lands as well as a summary of the extensive existing information available on Project area resources. The PAD also included ten draft study plans describing a subset of the Districts' proposed relicensing studies. The Districts then convened a series of Resource Work Group meetings, engaging agencies and other relicensing participants in a collaborative study plan development process culminating in the Districts' Proposed Study Plan (PSP) and Revised Study Plan (RSP) filings to FERC on July 25, 2011 and November 22, 2011, respectively.

On December 22, 2011, FERC issued its Study Plan Determination (SPD) for the Project, approving, or approving with modifications, 34 studies proposed in the RSP that addressed Cultural and Historical Resources, Recreational Resources, Terrestrial Resources, and Water and Aquatic Resources. In addition, as required by the SPD, the Districts filed three new study plans (W&AR-18, W&AR-19, and W&AR-20) on February 28, 2012 and one modified study plan (W&AR-12) on April 6, 2012. Prior to filing these plans with FERC, the Districts consulted with relicensing participants on drafts of the plans. FERC approved or approved with modifications these four studies on July 25, 2012.

Following the SPD, a total of seven studies (and associated study elements) that were either not adopted in the SPD, or were adopted with modifications, formed the basis of Study Dispute proceedings. In accordance with the ILP, FERC convened a Dispute Resolution Panel on April 17, 2012 and the Panel issued its findings on May 4, 2012. On May 24, 2012, the Director of FERC issued his Formal Study Dispute Determination, with additional clarifications related to the Formal Study Dispute Determination issued on August 17, 2012. The *Chinook Salmon Otolith Study* (W&AR 11) was not a subject of the dispute resolution process.

On January 17, 2013, the Districts issued the Initial Study Report (ISR) and held an ISR meeting on January 30 and 31, 2013. The Districts filed a summary of the ISR meeting with FERC on February 8, 2013. Comments on the meeting summary and requests for new studies and study modifications were filed by relicensing participants on or before March 11, 2013, and the

Districts filed reply comments on April 9, 2013. FERC issued the Determination on Requests for Study Modifications and New Studies on May 21, 2013. The determination did not involve the study plan for the *Chinook Salmon Otolith Study* (W&AR 11).

The Districts filed the Updated Study Report (USR) on January 6, 2014; held a USR meeting on January 16, 2014; and filed a summary of the meeting on January 27, 2014. Relicensing participant comments on the meeting summary and requests for new studies and study modifications were due by February 26, 2014. The Districts filed reply comments on March 28, 2014. FERC issued the Determination on Requests for Study Modifications on April 29, 2014.

This study report describes the objectives, methods, and results of the *Chinook Salmon Otolith Study* (W&AR 11) as implemented by the Districts in accordance with FERC's December 22, 2011 Order. Documents relating to the Project relicensing are publicly available on the Districts' relicensing website at: http://www.donpedro-relicensing.com/

2.0 CHINOOK SALMON OTOLITH STUDY GOALS AND OBJECTIVES

Otoliths (commonly referred to as "earstones") are calcium carbonate structures in the inner ear of fish that grow in proportion to the overall growth of the individual, such that daily or weekly growth increments can be measured to allow the age and fish size at various habitat transitions to be identified. Through analysis of otoliths, the goal of this study was to identify the geographic origin and early life history rearing and emigration patterns of Tuolumne River Chinook salmon during above- and below-normal water year (WY) types. Examination of otolith microstructure has been used to identify differing rearing environments of juvenile salmon (e.g., Neilson et al. 1985) as well as differences in rearing temperatures (Zhang et al. 1995; Volk et al. 1996). Additionally, using one of several methods of microchemical analysis, the concentrations of elements (e.g., strontium, barium, calcium) and proportions of stable strontium (Sr) isotopes in otoliths may be compared to those in the water in which the fish inhabits in order to provide a tracer of the location where the fish has been (e.g., freshwater, saltwater, natal stream) (Campana and Neilson 1985). Otolith microchemistry has been used to examine early life history rearing environments of salmonids to address questions of streams of natal origin (Ingram and Weber 1999; Campana and Thorrold 2001) as well as the timing of entry into estuarine and saline environments (Zimmerman 2005).

This study applies microstructural and microchemical analysis of otoliths to address questions regarding the success of various early life-history emigration patterns of fall-run Chinook salmon originating from the Tuolumne River. Early life history events in juvenile salmonid development, including incubation, emergence, and habitat transitioning, can be linked to otolith microstructural patterns due to the thermal, physical, and chemical regime under which these fish were reared. Identification of the natal streams of adults that spawn in the Tuolumne River may allow additional quantification of straying rates from other rivers and, hence, more accurate assessments of the population size of indigenous Tuolumne River salmon. The relative contribution of emigrant fry, parr and smolts to subsequent escapement may have implications for the magnitude and timing of flow in the Tuolumne River, as well as the timing of operations of barriers and export facilities in the southern Sacramento and San Joaquin River delta (Delta[1]).

In brief, the study objectives were to use otolith microstructural growth patterns and/or microchemistry in order to identify:

- whether returning adults originated from hatcheries or riverine environments other than the Tuolumne River; and,
- growth rates and sizes of 'wild' fish at exit from the Tuolumne River and from the freshwater Delta.

[1] The Delta received its first official boundary in 1959 with the passage of the Delta Protection Act (Section 12220 of the California Water Code), with the southern boundary in the San Joaquin River located at Vernalis (RM 69.3) and a western boundary at the confluence of the Sacramento and San Joaquin Rivers (RM 0) near Chipps Island.

3.0 STUDY AREA

The study area consists of locations of Chinook salmon carcass recoveries collected by California Department of Fish and Wildlife (CDFW) from the lower Tuolumne River, typically extending from approximately 0.5 miles downstream of the lower end of the La Grange powerhouse tailrace (RM 51.6) to the end of routine spawning surveys at approximately RM 21.2. The lower San Joaquin River from the Tuolumne River confluence (RM 84) to Vernalis (RM 69.3), Delta, San Francisco Bay Estuary[2], and the Pacific Ocean are also addressed in terms of their use by rearing and outmigrant juvenile life stages of Chinook salmon.

[2] The greater San Francisco Bay estuary extends from the Golden Gate Bridge in San Francisco Bay eastwards across salt and brackish water habitats included in San Leandro, Richardson, San Rafael, and San Pablo bays, as well as the Carquinez Strait, Honker, and Suisun bays further to the east near the western edge of the Delta.

4.0 METHODOLOGY

4.1 Existing Data Compilation

This study relied upon the existing inventory of fall-run Chinook salmon otoliths sampled from unmarked carcasses collected by CDFW during annual spawner escapement surveys in the lower Tuolumne River, which are typically conducted from October to early-January. Otoliths were provided cooperatively by CDFW under a memorandum of understanding (MOU) with the Districts and the Department of Animal Science, University of California, Davis (UC Davis). In order to examine potential variations in early life-history emigration patterns, otoliths were selected to represent returning adults that had outmigrated during five focus years (1998, 1999, 2000, 2003, and 2009), representing “above normal” or “wet” and “below normal” or “dry” WY types[3]. With a sampling goal of obtaining 100–200 otoliths from each outmigration year for laboratory analysis, these five years were also selected because they represented years with the greatest number of available samples from the existing CDFW inventory. The sampling goal was met for the above normal/wet WY types 1998, 1999, and 2000, but was not met for the below normal/dry WY types 2003 and 2009, which had comparatively fewer samples available (Table 4.2-1). As the otoliths were collected from unmarked fish, the samples did not include known hatchery-origin fish[4].

4.2 Laboratory Otolith Analysis

A summary of the otolith analytical methods is provided below, with additional details provided in Sturrock and Johnson (2014) (see Attachment A).

4.2.1 Adult sampling and cohort reconstruction

Adult salmon from a given outmigration year typically return between 2 and 5 years later with the greatest proportion returning after 3 and 4 years respectively in historical Tuolumne River spawner surveys (TID/MID 2014a). Thus, for each outmigration year that was examined in this study, otolith samples were recovered from carcasses collected over several escapement years (Table 4.2-1). Experts at CDFW determined the ages of the adult samples by counting scale winter annuli from unmarked adult salmon carcasses in accordance with established and validated techniques (Guignard 2008). Information regarding the date of collection, location, fish length, sex, and estimated age-at-return were provided by CDFW for each otolith sample.

[3] CDWR Bulletin 120 estimates unimpaired runoff as TAF for the San Joaquin River and tributaries. The San Joaquin Basin 60-20-20 Index classifies water years (October 1 through September 30) into five basic types (C=Critical, D=Dry, BN=Below Normal, AN=Above Normal, W=Wet) which are further refined under Article 37 of the FERC (1996) license. For the purposes of this report, the broader CDWR Water Year types are used as a basis of discussion.

[4] Although the Merced River Fish Facility (MRFF) does not participate in the Constant Fractional Marking Program implemented since 2007, the MRFF historically only marked a proportion of hatchery fish, and that proportion has varied over time.

Table 4.2-1. Otolith sampling inventory by juvenile cohort and outmigration WY type collected from unmarked adult salmon carcasses in the Tuolumne River between 1999 and 2012. Source: Sturrock and Johnson (2014) (see Attachment A).

Juveniles Represented			Adults Sampled			
Spawning year[1]	Outmigration year[2]	WY type during rearing & outmigration[3]	Escapement year[4]	Estimated age at return (yr)[5]	Number of individuals sampled	% of total sample
1997	1998	Wet	1999	2	0	0%
			2000	3	124	62%
			2001	4	76	38%
			Sum		**200**	**100%**
1998	1999	Above normal	2000	2	9	6%
			2001	3	64	44%
			2002	4	73	50%
			Sum		**146**	**100%**
1999	2000	Above normal	2001	2	31	28%
			2002	3	79	72%
			2003	4	0	0%
			Sum		**110**	**100%**
2002	2003	Below normal	2004	2	0	0%
			2005	3	87	91%
			2006	4	9	9%
			Sum		**96**	**100%**
2008	2009	Below normal	2010	2	14	30%
			2011	3	30	65%
			2012	4	2	4%
			Sum		**46**	**100%**
TOTAL					**598**	

1 Although CDFW uses the term "brood-year" to designate the year in which fry first emerge (typically December), here we simply indicate the year in which the majority of spawning occurred.
2 Outmigration-year designation is based on the timing of the first juveniles' departure from the natal river.
3 CDWR Bulletin 120 estimates unimpaired runoff as TAF for the San Joaquin River and tributaries. The San Joaquin Basin 60-20-20 Index classifies WYs (October 1 through September 30) into five basic types (C=Critical, D=Dry, BN=Below Normal, AN=Above Normal, W=Wet), which are further refined under Article 37 of the FERC (1996) license. For the purposes of this report, the broader CDWR WY types are used as a basis of discussion.
4 Sampled during CDFW annual spawner escapement surveys.
5 Estimated from CDFW scale readings.

4.2.2 Strontium isotope analysis

Adult otoliths were prepared and analyzed for strontium isotopic ($^{87}Sr/^{86}Sr$) ratios using standard techniques described in Sturrock and Johnson (2014) (see Attachment A). In brief, the technique relies on detecting daily deposition of chemical elements from the surrounding environment in otolith growth rings, producing a distinct and reproducible "chemical fingerprint". In the California Central Valley, strontium isotopes ($^{87}Sr/^{86}Sr$) are ideal markers because the water signature varies with watershed geology, therefore differing among many of the rivers and salmon outmigration paths (Barnett-Johnson et al. 2008, Ingram and Weber 1999).

Otoliths were rinsed and cleaned of adhering tissue, then mounted in resin and polished until each primordial core (i.e., center) was exposed. Each otolith was sampled at multiple spots along a 90° radial transect starting at the primordial core and ending just past the point of ocean entry (also called the "freshwater exit"), in order to ensure inclusion of the full freshwater outmigration period in the analysis (Figure 4.2-1). At each sample spot, $^{87}Sr/^{86}Sr$ ratios were determined by multi-collector laser ablation inductively coupled plasma mass spectrometry (MC-LA-ICPMS) (Barnett-Johnson et al. 2005). To improve the spatial resolution and accuracy of the ocean entry spot identification and outmigration fork length (see also Section 4.2.4), additional $^{87}Sr/^{86}Sr$ sample spots were re-sampled at the region representing an isotope ratio shift (e.g., the Tuolumne-San Joaquin River transition).

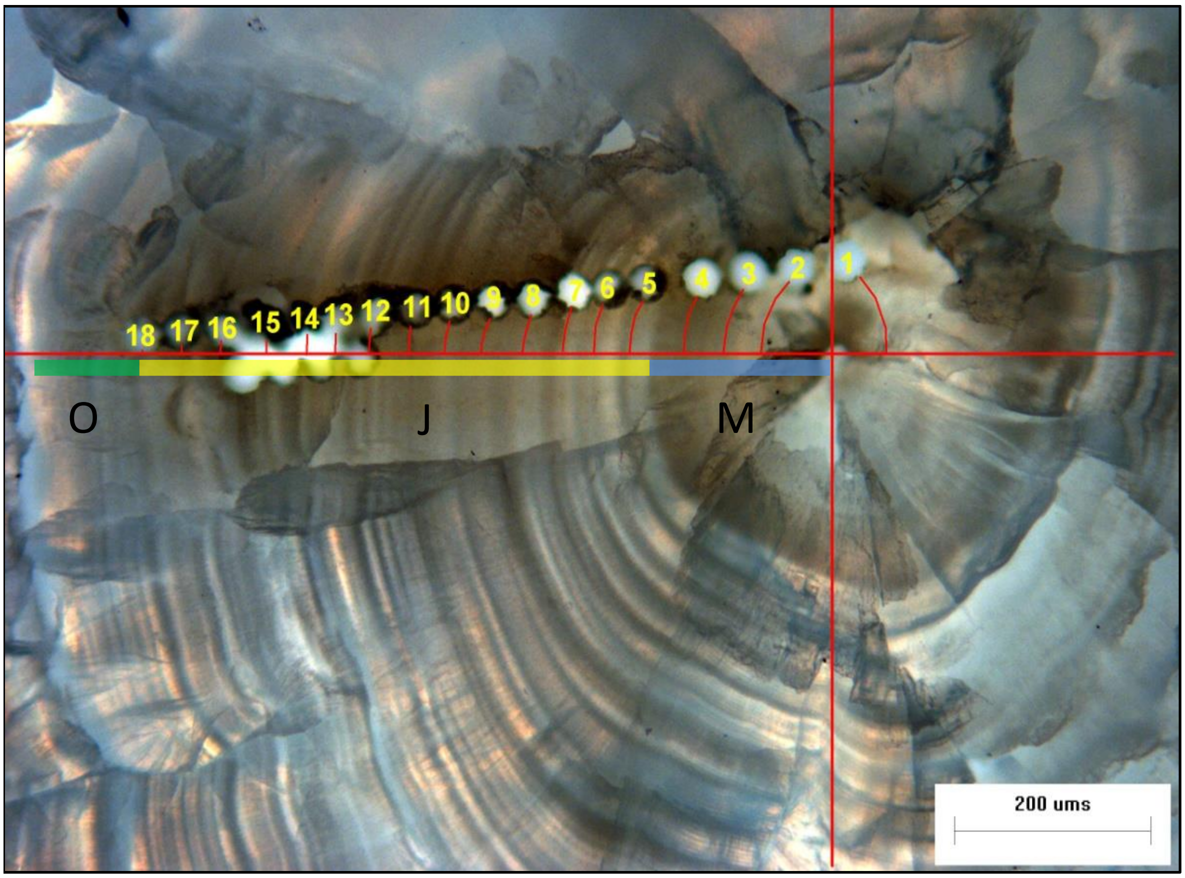

Figure 4.2-1. A typical $^{87}Sr/^{86}Sr$ transect showing spot analyses (numbered) from the core to ocean entry. The life history stages are indicated by letters: maternal (M), juvenile (J) and ocean (O). The distance at which the final 'natal spot' intersected the 90° transect (indicated by curved red lines) was used to back-calculate size at outmigration. 'Respots' occurred at positions 12.5 to 15.5 used to more accurately identify exit point. Source: Sturrock and Johnson (2014) (see Attachment A).

4.2.3 Identification of natal origin

To identify the natal origin of the otolith samples, measured $^{87}Sr/^{86}Sr$ ratios were statistically compared to a "strontium isoscape" comprised of the previously published $^{87}Sr/^{86}Sr$ baseline for California Central Valley rivers and hatcheries, additional Sr isotope values of otolith samples from juveniles and coded wire tag (CWT) adults known to originate from the Tuolumne River, and Sr isotope values from Tuolumne River and San Joaquin River water samples collected in 2014 (A. Sturrock, unpublished; Ingram and Weber 1999; P. Weber, unpublished). The resulting strontium isoscape included a total of 480 tissue and water samples from all potential natal sources in the California Central Valley, with many sites sampled across multiple years (1998–2013) and hydrologic regimes (Sturrock and Johnson 2014) (see Attachment A, Table 3).

Given the variability in Sr isotope values in water samples from upper to lower reaches of the lower Tuolumne River (A. Sturrock, unpublished; Ingram and Weber 1999; P. Weber, unpublished), juveniles collected in the Tuolumne River tend to exhibit more variable isotopic signatures within and among individuals than in other rivers in the Central Valley (Figure 4.2-2). Additionally, otolith $^{87}Sr/^{86}Sr$ values of known-origin Tuolumne River fish, Mokelumne River Hatchery and Feather River Hatchery can overlap (Figure 4.2-2), increasing the potential of misclassifying Tuolumne-origin fish. To improve assignment accuracy, any otolith samples exhibiting ambiguity in their natal assignment were also analyzed for otolith microstructural features that can discriminate hatchery from wild fish. Following methods developed for California Central Valley Chinook (Barnett-Johnson et al. 2007), individuals were classified as hatchery or wild based on the prominence of the exogenous feeding check (scored blind by 2–3 independent readers) and the mean and variance in increment width around the first 30 daily increments following onset of exogenous feeding after fry emergence from the spawning gravels.

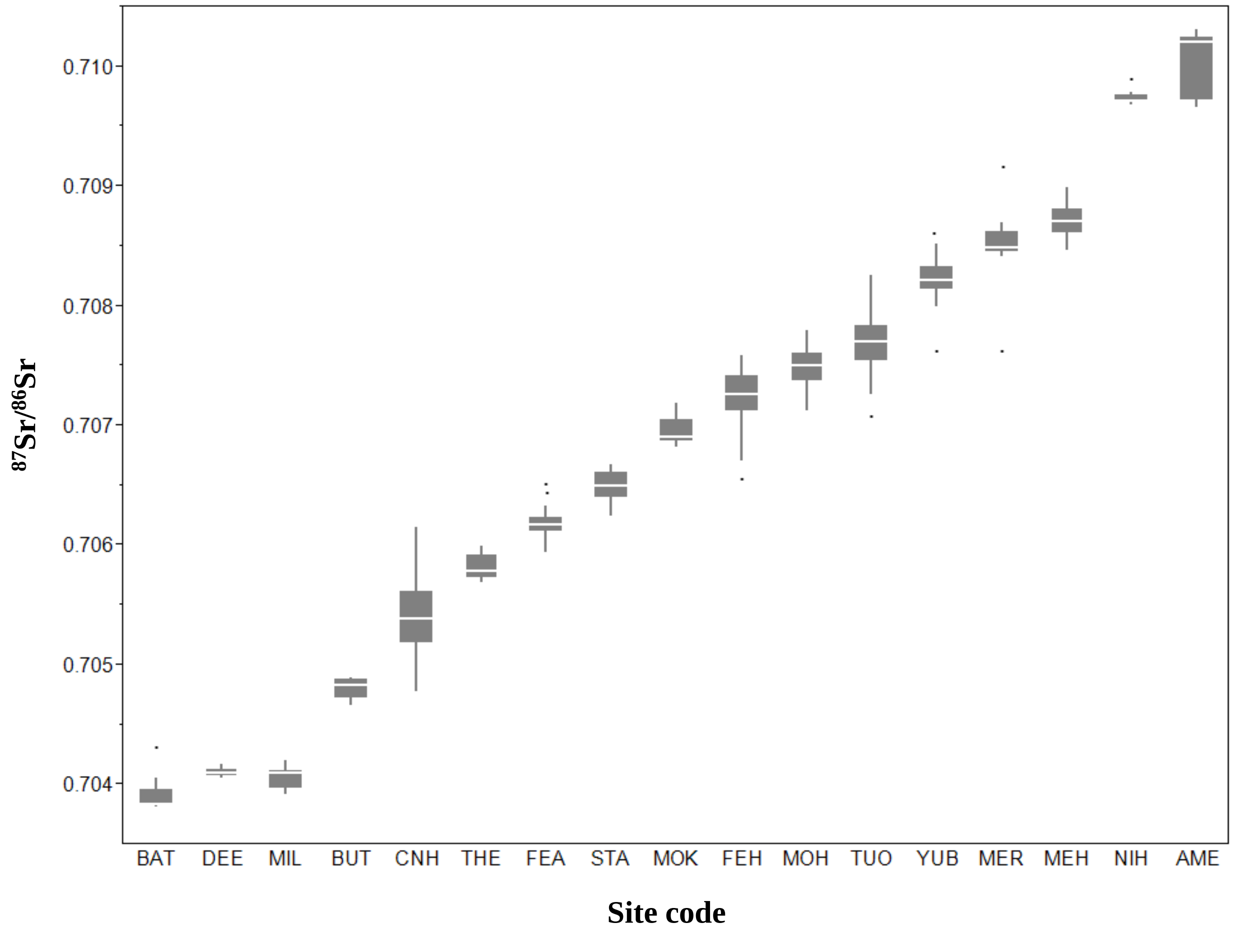

Figure 4.2-2. Differences in $^{87}Sr/^{86}Sr$ values among sites in the California Central Valley. Due to overlap among the Tuolumne River (TUO), Mokelumne River Hatchery (MOH), and Feather River Hatchery (FEH), all fish identified as potentially originating from the Tuolumne River using Sr isotopes were also assigned to hatchery/wild using otolith microstructure. Other side codes: Battle Creek (BAT), Deer Creek (DEE), Mill Creek (MIL), Butte Creek (BUT), Coleman National Fish Hatchery (CNH), Thermalito Rearing Annex (THE), Feather River (FEA), Stanislaus River (STA), Mokelumne River (MOK), Yuba River (YUB), Merced River (MER), Merced River Hatchery (MEH), Nimbus Hatchery (NIH), American River (AME). Source: Sturrock and Johnson (2014) (see Attachment A).

4.2.4 Reconstructing size and age at outmigration

Variations in the $^{87}Sr/^{86}Sr$ ratio along the sampling transect were used to indicate the location and thus life history timing of emigration from the Tuolumne River ('natal exit') using the distance from the otolith primordial core to the 'last natal spot'. The 'last natal spot' rather than the 'first non-natal spot' was used because to accrete sufficient new otolith material to modify the isotopic composition of the otolith, the fish would have inhabited isotopically distinct (i.e., non-natal) water for several days, after which time it would be a significant distance downstream of the Tuolumne-San Joaquin River confluence. The 'last natal spot' was identified by working backwards from the final inflection point indicative of ocean-bound migration, and using the spot just prior to the lowest point of inflection, where the latter represented likely movement through the San Joaquin River (see Attachment A, Fig. 3A-C). The only exceptions were on occasions when the lowest point prior to ocean migration was lower than any value measured in the San Joaquin River (see Attachment A, Fig. 3D); on these occasions the lowest point was assumed to have been deposited while the fish was rearing in the lower Tuolumne River, which has been shown to exhibit $^{87}Sr/^{86}Sr$ values as low as 0.7066 (see Attachment A).

The point of emigration from freshwater (‘freshwater exit’) was defined as the distance at which otolith $^{87}Sr/^{86}Sr$ values last reached 0.7080 (equivalent to a salinity of 1ppt based on Hobbs et al. 2010), determined using linear interpolation.

In order to estimate fish size at the natal and freshwater exit points, radial otolith distances to these points were measured for use with an existing relationship between otolith radius and fork length (FL) from the California Central Valley fall run Chinook salmon Evolutionarily Significant Unit (ESU) (Zabel et al. 2010). Juvenile reference samples for the Zabel et al. (2010) relationship were collected at various locations including samples from the Tuolumne River (2003; n = 6), Stanislaus River (2000 and 2002; n = 95), the Coleman National Fish Hatchery (2002; n=40) and in the San Francisco Bay at Golden Gate Bridge (2005; n = 83) (Figure 4.2-3). While the small number of Tuolumne-origin fish included in the relationship tended to sit above the mean regression line (Figure 4.2-3), there was no significant difference between the back-calculated fork length of Tuolumne vs. non-Tuolumne fish, nor any difference in the slopes (see Attachment A). The uncertainty in the otolith radius-fork length regression was used to estimate 95% confidence intervals (CI) for the estimated juvenile fork lengths associated with individual adult otolith samples.

For each length estimate at natal exit from the Tuolumne River, fish were classified as fry (<50 mm FL), parr (≥50 to <70 mm FL), and smolt (≥70 mm FL) in this report. Although these size cutoffs are 5 mm larger than those from the Mokelumne River (Miller et al. 2010) used in Attachment A, the Tuolumne River size classes were re-assigned here based upon operational definitions used in juvenile outmigration studies (TID/MID 2014b). For example, the smallest sized juveniles reported as smolts in historical sampling range as low as 65 mm FL in some years (Stillwater Sciences 2013a).

Fish age at outmigration was determined by counting daily growth bands and measuring widths between daily increments along the same 90° radial transect as the $^{87}Sr/^{86}Sr$ analysis, beginning at the point when the maternal yolk sac is depleted and exogenous feeding begins (“post exogenous feeding check”) until freshwater exit from the Delta to the San Francisco Bay and Pacific Ocean. Some otoliths were difficult to age and given low readability scores (1-2); ages were not provided for these individuals. The ages of fish at natal exit from the lower Tuolumne River, freshwater exit from the Delta, and habitat-specific growth rates were obtained for fish with otolith readability scores of 3–5. A subset of otoliths was aged by two independent readers, providing an estimate of error associated with fish aging. The two independent reads of each fish demonstrated high agreement, with an average difference of ± 5 days (range 0–12 days).

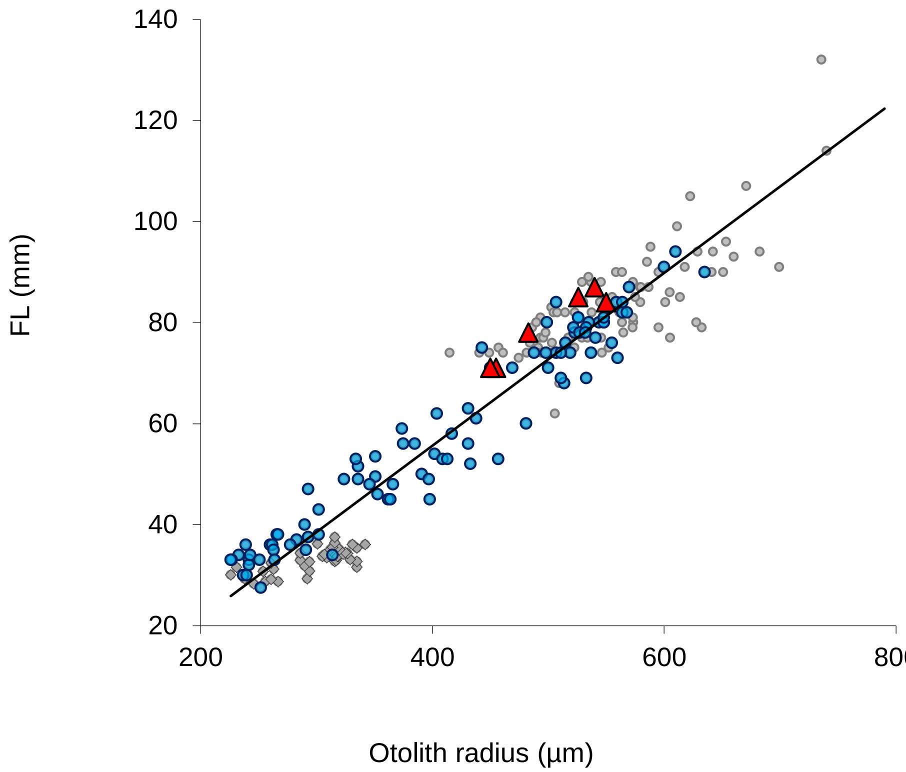

Figure 4.2-3. Relationship between otolith radius and fork length (FL) of juveniles of known origin from the California Central Valley fall run Chinook salmon Evolutionarily Significant Unit (ESU). (n=224, r^2 = 0.92) Red triangles = Tuolumne River (n = 6); blue circles = Stanislaus River (n = 95); grey diamonds = Coleman National Fish Hatchery (n=40); grey circles = San Francisco Bay at Golden Gate Bridge unknown origin (n = 83). Source: Sturrock and Johnson (2014) (see Attachment A).

4.3 Analysis of Potential Flow Relationships

Tuolumne River hydrologic patterns were explored for each of the five outmigration years using available flow data for gages at La Grange (USGS #11289650), Modesto (USGS #11290000), and Vernalis (USGS #11303500). Daily flow data were pooled to develop flow metrics at 2-week and monthly intervals from January through June, including minimum, maximum, and mean Tuolumne River discharge. Each of the Tuolumne River flow metrics were used in linear regressions against fish size at natal exit and fish age at natal exit (determined by the otolith analyses) for each of the five outmigration years included in the study (1998, 1999, 2000, 2003, and 2009).

Average daily flow magnitude and timing were also examined in combination with mean fish size and age at exit from the Tuolumne River and the Delta to determine any potential relationships between flow and fish age/size at exit. This exploratory analysis was undertaken to determine whether flow may explain various early life-history emigration patterns of juvenile salmon from differing WY types.

Delta hydrologic patterns were investigated using California Department of Water Resources (CDWR) DAYFLOW data, including 24 flow parameters and indices characterizing the following (DWR 2011):

- daily river inflows (e.g., Sacramento, Yolo, Cosumnes, Mokelumne, San Joaquin, Calaveras plus other miscellaneous creek flows);
- interior Delta flows (e.g., Delta Cross Channel and Georgiana Slough, Jersey Point, Rio Vista);
- water exports and diversions/transfers (e.g., Central Valley Project at Tracy, Contra Costa Water District Diversions at Middle River, Rock Slough, Old River, North Bay Aqueduct, State Water Project);
- estimates of Delta agriculture depletions; and,
- fish-related flows (i.e., percent water diverted, effective Western/Central Delta inflow, effective percent Western/Central Delta water diverted).

Daily average flow data for each of the DAYFLOW 24 parameters/indices were pooled into aggregated monthly averages from January through June. Each of these averages were used in exploratory linear regressions against fish size at freshwater exit and fish age at freshwater exit for each of the five outmigration years included in the study (1998, 1999, 2000, 2003, and 2009).

5.0 RESULTS

5.1 Natal Origin

Analysis of Sr isotope ratios ($^{87}Sr/^{86}Sr$) and otolith microstructural features (see Section 4.2.3) in the unmarked fish samples indicated both wild- and hatchery-origin fish in Tuolumne River spawning adults corresponding to outmigration years 1998, 1999, 2000, 2003, and 2009 (Figure 5.1-1). The earliest three years exhibited the highest numbers of Tuolumne River returning wild fish, with smaller numbers of wild fish exhibiting Sr isotope ratios indicating straying from the Stanislaus, Merced, and Mokelumne rivers. The hatchery component in these outmigration years was primarily from the Merced and Mokelumne river hatcheries, with smaller contributions from the Feather River and Nimbus hatcheries. Overall, returning wild fish made up 38–68% of the sample of unmarked fish for outmigration years 1998–2000 (

Table 5.1-1). During outmigration years 2003 and 2009, relatively low numbers of returning wild fish were present in the sample, with larger hatchery components primarily from the Mokelumne River Hatchery (2003) and the Coleman National Fish Hatchery (2009) (

Table 5.1-1). Overall, returning wild fish made up 9–25% of the sample for outmigration years 2003 and 2009 (

Table 5.1-1). Considering all five outmigration years combined (n=598), 54% of the unmarked fish samples were identified as wild and of Tuolumne River origin (n=321), 43% were identified as hatchery-origin (n=255), and 4% were identified as wild strays from other rivers (n=22).

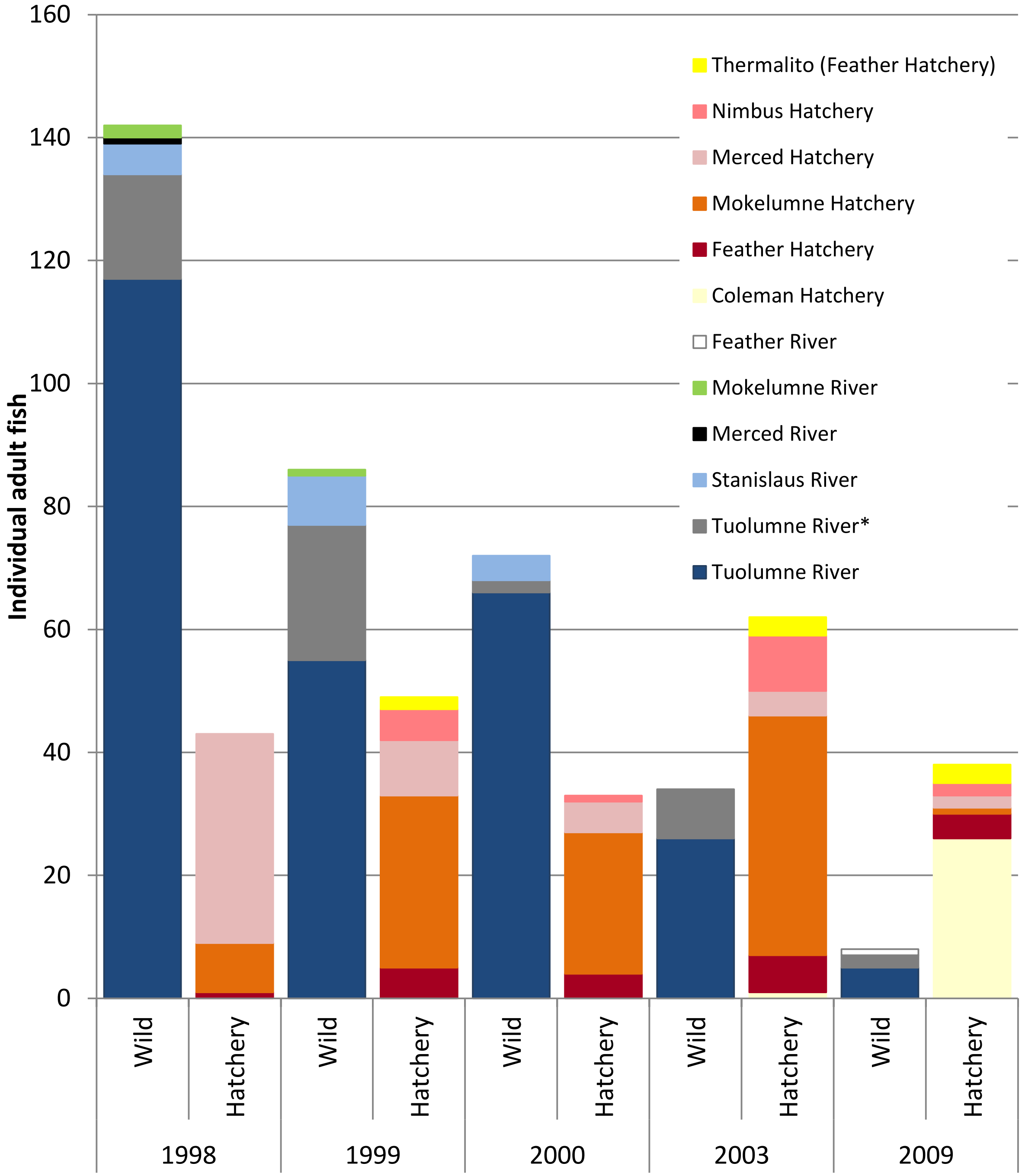

Figure 5.1-1. Natal origin of all unmarked fish (n=598) analyzed for outmigration years 1998, 1999, 2000, 2003 and 2009. * indicates individuals assigned to the Tuolumne River with <0.5 posterior probability based on mean natal $^{87}Sr/^{86}Sr$ values or individuals assigned to the Tuolumne River, but with inconclusive hatchery/wild assignment based on otolith microstructure. Data from Sturrock and Johnson (2014) (see Attachment A).

Table 5.1-1. Summary of straying and return rates to the Tuolumne River for unmarked fish (n=598). Data from Sturrock and Johnson (2014) (see Attachment A).

Outmigration year	San Joaquin River Index Water Year Type[1]	Sample size	Returns (Wild)[2]	Strays (Wild and Hatchery)[2]	Primary origin of strays
1998	Wet	200	57–68%	33–44%	Merced Hatchery
1999	Above normal	146	38–53%	47–62%	Mokelumne Hatchery
2000	Above normal	110	61–64%	36–39%	Mokelumne Hatchery
2003	Below normal	96	27–35%	65–73%	Mokelumne Hatchery
2009	Below normal	46	9–15%	85–91%	Coleman Hatchery

[1] San Joaquin Basin 60-20-20 Index from CDWR Bulletin 120.
[2] Range in natal assignment is based on probabilities associated with the isotope-based discriminant function analysis and reference samples from existing or ongoing projects.

5.2 Growth and Residency of Juveniles

Mean fish size at exit from the Tuolumne River ranged 63.5–76.0 mm, with the lowest mean size exhibited in outmigration year 2000. The year 2000 mean size was significantly different ($p<0.005$) from that exhibited in the other four years of the study. Similarly, age at exit from the Tuolumne River was lower in outmigration year 2000 (68.5 days) as compared with that of other years, although there was generally higher variability in age at exit such that no single year was statistically lowest. Tuolumne River growth rates were similar across all years, with the highest rates and the greatest variability exhibited in 2003 (Table 5.2-1).

Mean fish size at freshwater exit from the Delta ranged 77.4–83.4 mm, with slightly greater variability within years than that of the Tuolumne River (Table 5.2-1). Examination of the distributions of age at exit from the Tuolumne River and the Delta suggests that overall the total days from the end of exogenous feeding (i.e., emergence from gravels) to ocean entry was relatively constant at 99±20 days for each of the five outmigration years, such that fewer days spent rearing in the Tuolumne River resulted in relatively more days rearing in the Delta (Figure 5.2-1). Estimated growth rates were generally greater in the Delta than in the Tuolumne River for corresponding outmigration years, with the exception of 2009. Variability in growth rates was also greater in the Delta (Table 5.2-1).

Table 5.2-1. Summary of fish size, age, and growth rates (mean ±1SD) at natal exit and freshwater exit by outmigration year for juveniles that originated in and returned to the Tuolumne River. Source: Sturrock and Johnson (2014) (see Attachment A).

Out-migration year (WY Type[2])	Sample Size	Tuolumne River			Delta		
		FL at exit (mm)	No. increments (days)	Increment width[1] (um)	FL at exit (mm)	No. increments (days)	Increment width[1] (um)
1998 (W)	117	73.3 ± 8.5	91.0 ± 16.2	3.07 ± 0.28	80.8 ± 9.0	15.8 ± 7.5	3.24 ± 0.54
1999 (AN)	55	72.6 ± 11.6	82.0 ± 13.6	3.20 ± 0.27	82.3 ± 11.5	16.5 ± 8.7	3.35 ± 0.56
2000 (AN)	66	63.5 ± 8.6	68.5 ± 18.6	3.10 ± 0.26	77.4 ± 6.9	27.6 ± 12.1	3.52 ± 0.52
2003 (BN)	26	71.0 ± 10.6	79.7 ± 17.9	3.39 ± 0.43	80.1 ± 10.0	10.5 ± 5.2	3.65 ± 0.62
2009 (BN)	5	76.0 ± 7.1	88.0 ± 20.3	3.36 ± 0.29	83.4 ± 6.8	16.0 ± 7.5	3.03 ± 0.36

1 Width between daily increments is a measure of growth rate.
2 San Joaquin Basin 60-20-20 Index from CDWR Bulletin 120.

Using typical size classes for juvenile outmigrants from the Tuolumne River (fry <50 mm FL, parr ≥50 to <70 mm FL, and smolt ≥70 mm FL), all size classes were represented in the adult spawning population. However, Tuolumne-origin adults were overwhelmingly comprised of individuals that had emigrated from the Tuolumne as parr and smolts, with only small fry contributions evident in 2000 and 2003 (Table 5.2-2). In 2000, a relatively high percentage of the returning adults outmigrated as parr (70%). In 2009, although the sample size was very low (n=5), an apparently high percentage of the returning adults outmigrated as smolts (80%) (Table 5.2-2).

Table 5.2-2. Water year type and juvenile outmigrant size classes at natal exit for unmarked fish. Size classes revised from fork length data presented in Sturrock and Johnson (2014) (see Attachment A).

Outmigration year	San Joaquin River Index Water Year Type	N	Fry (< 50 mm)	Parr (50–69 mm)	Smolt (≥ 70 mm)
1998	Wet	117	0%	34%	66%
1999	Above normal	55	0%	38%	62%
2000	Above normal	66 [a]	5%	70%	26%
2003	Below normal	26	4%	42%	54%
2009	Below normal	5	0%	20%	80%

[a] Sample size for outmigration year 2000 incorrectly reported as 67 in Sturrock and Johnson (2014) (see Attachment A).

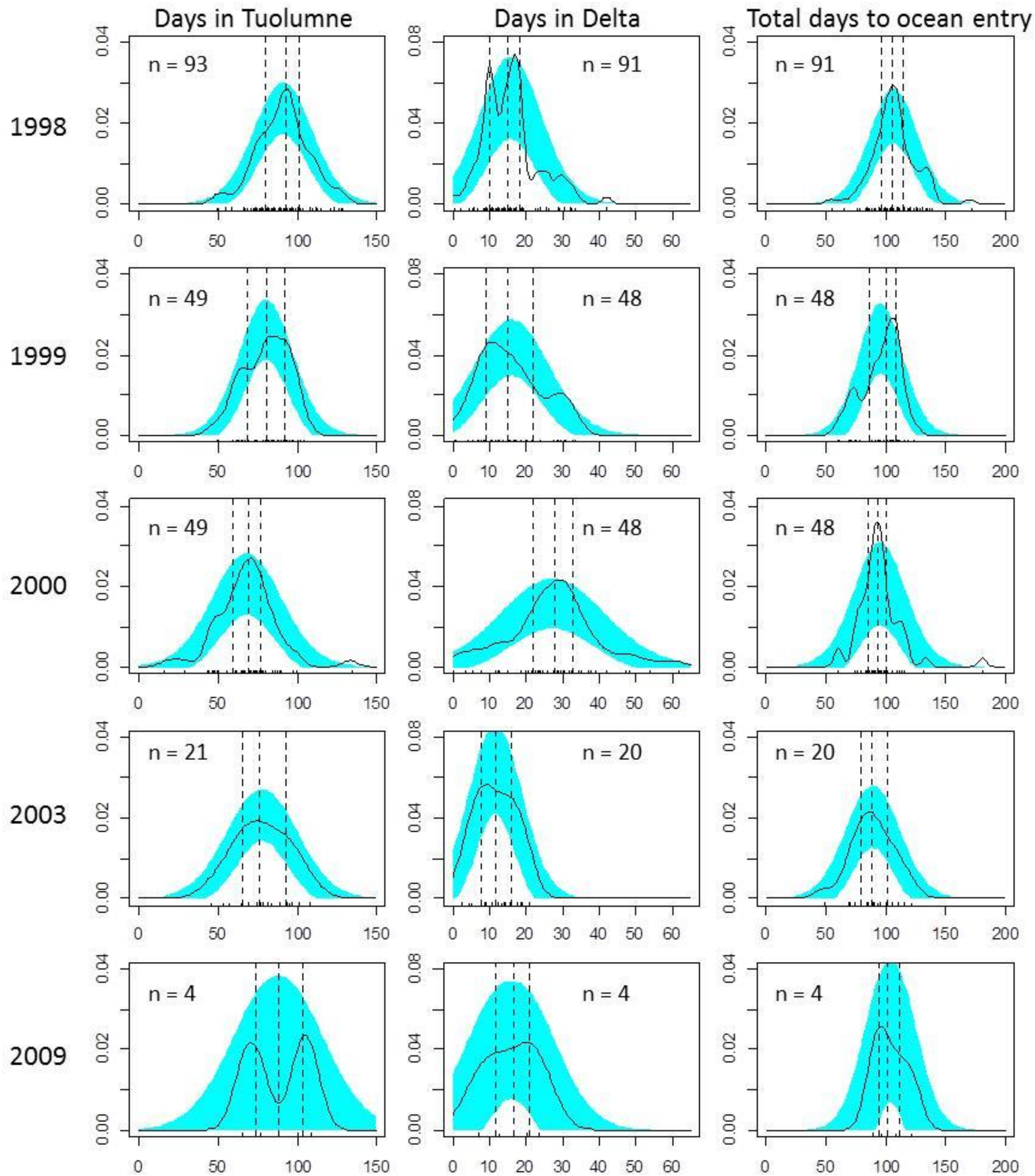

Figure 5.2-1. Days of development from formation of otolith core to ocean entry. The rug plots show values for individual otoliths from unmarked adult samples. The curves are non-parametric density estimates obtained by kernel smoothing, deliberately under-smoothed. The cyan bands encode a test for normality. The vertical dashed lines mark the data quartiles.

5.3 Hydrology

5.3.1 Daily flows

Tuolumne River hydrographs for WYs 1998, 1999, 2000, 2003, and 2009 are presented in Figure 5.3-1 and Figure 5.3-2. At the La Grange and Modesto gages, during the three above normal/wet WY types (1998, 1999, 2000), winter flows increased during December through February, typically remaining at or above 2,000 cfs until at least early/mid-summer. In WY 1998, average daily flows increased beginning in mid-January and remained high, exceeding 5,000 cfs multiple times from February through July. In WY 1999, flows increased to 2,000-3,000 cfs in December, and again in mid-January, remaining generally at or near this range through mid-May. WY 2000 experienced a relatively later increase in winter flows than either WY 1998 or 1999, with flow increases occurring in mid-February (Figure 5.3-1, Figure 5.3-2).

Average daily flows at La Grange during the two below normal/dry WY types (2003, 2009) remained at or below approximately 200 cfs through March, with pulse flow releases peaking in mid-April at 1,500 cfs in WY 2003, and peaking in mid-May at 950 cfs in WY 2009 (Figure 5.3-1). In general, average daily flows were slightly greater further downstream at Modesto, with the exception of a short but relatively large increase in average daily flow (> 1,000 cfs) that occurred during early March in WY 2009 (Figure 5.3-2).

In the San Joaquin River at Vernalis, peak flows during the above normal/wet WY types 1998 and 1999 occurred in mid-February, although their relative magnitudes were opposite those of the Tuolumne River, with 1999 flows exceeding 1998 flows at this location (Figure 5.3-3). WY 2000 flows peaked approximately a month later in mid-March, consistent with hydrology exhibited in the Tuolumne River (Figure 5.3-1 and Figure 5.3-2). Average daily flows at Vernalis for the below normal/dry WY types exhibited the pulse flow releases in mid-April, similar to the Tuolumne River (Figure 5.3-3).

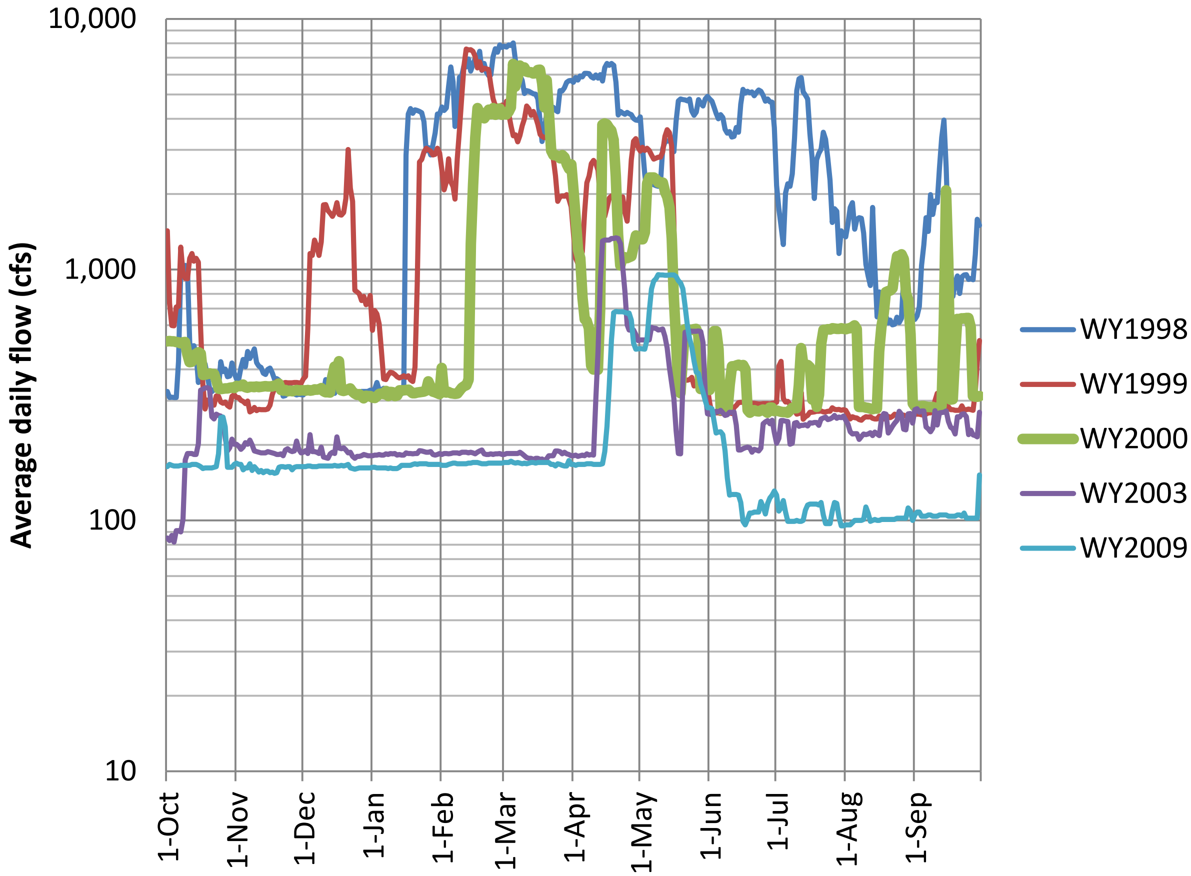

Figure 5.3-1. Tuolumne River average daily flow (cfs). Data from Tuolumne River Below La Grange Dam (USGS gage #11289650).

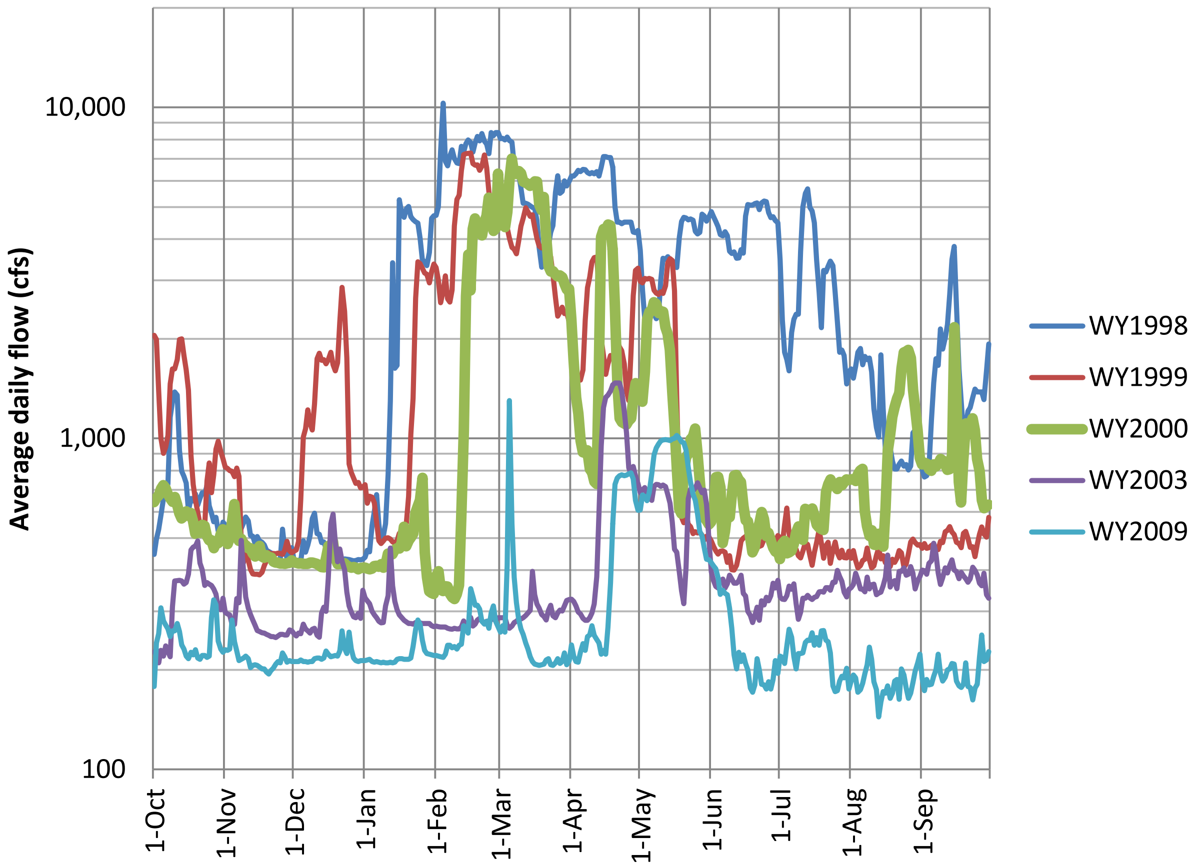

Figure 5.3-2. Tuolumne River average daily flow (cfs). Data from Tuolumne River at Modesto (USGS gage #11290000).

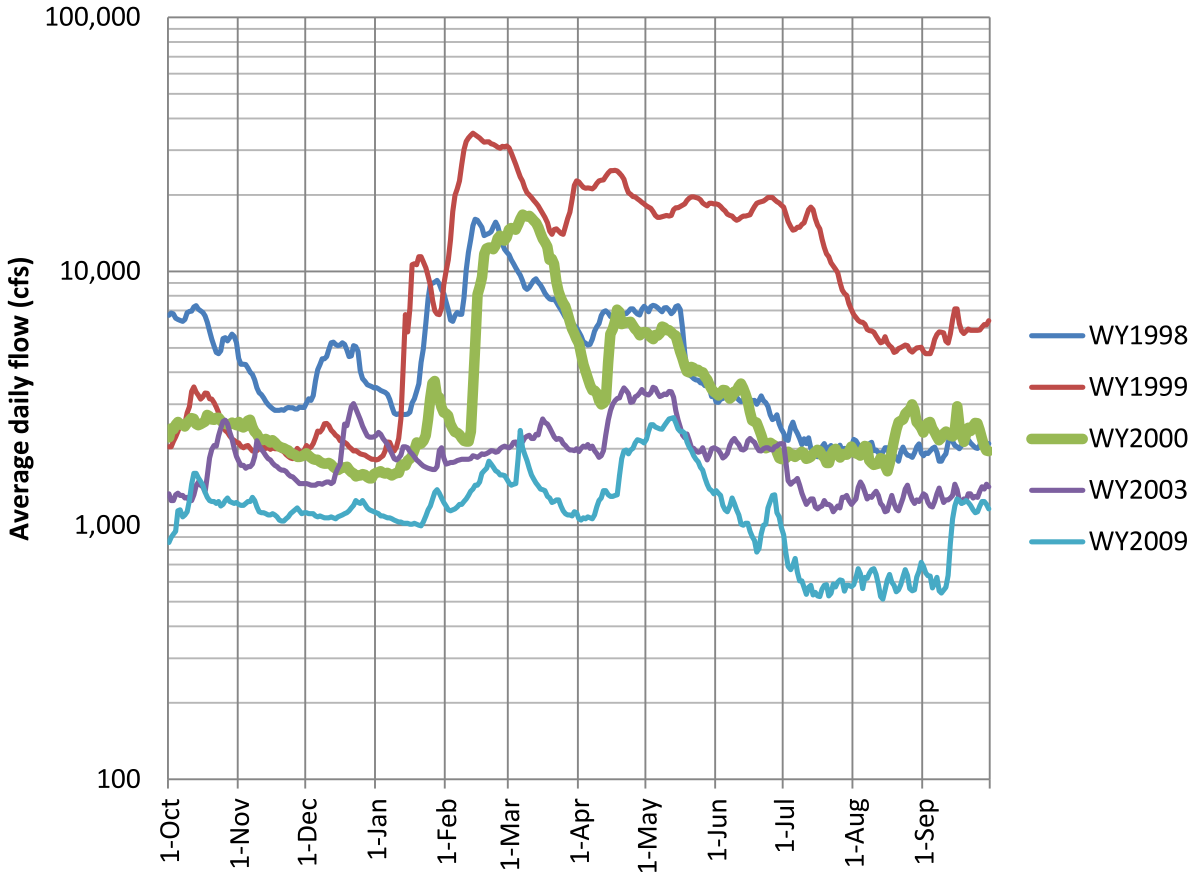

Figure 5.3-3. San Joaquin River average daily flow (cfs). Data from San Joaquin River at Vernalis (USGS gage #11303500).

5.3.2 Relationship between average daily flows and juvenile growth and residency

Within the above normal/wet WY types (1998, 1999, 2000), average daily flow magnitude and timing was examined in relation to mean fish size and age at exit for both the Tuolumne River (at La Grange and Modesto) and the Delta (at Vernalis). In 1998 and 1999, when average daily flows were sustained at relatively high levels during winter through spring months (extending into summer months in 1999), mean fish size and age at exit from the Tuolumne River were also relatively high, at approximately 73 mm FL (both years), 91 days (1998), and 82 days (1999) (Table 5.2-1). Although this pattern is consistent with prior observations of relatively larger sizes at emigration for above normal and wet WY types (Stillwater Sciences 2013b), mean fish size and age at natal exit were relatively lower at 64 mm and 69 days (Table 5.2-1) for outmigration year 2000, with the majority of individuals (70%) classified as parr (**Error! Reference source not found.**). In contrast to other above normal and wet WY types examined, daily flows in the Tuolumne River did not increase until later in the winter (mid-February) in 2000, and were generally sustained through mid-May.

Similar fish size associations were evident in the Delta as found at exit from the Tuolumne River, with larger mean fish size exhibited in outmigration years 1998–1999 than in 2000.

However, the mean number of days spent rearing in the Delta was roughly twice as high in 2000 as in 1998 and 1999. As noted previously (Section 5.2), overall the total days from the end of exogenous feeding (i.e., emergence from gravels) to ocean entry was relatively constant at 99±20 days across all outmigration years included in the study, such that fewer days spent rearing in the Tuolumne River resulted in relatively more days rearing in the Delta (Figure 5.2-1).

Within the below normal WY types (2003, 2009), when average daily flow increases did not occur until mid-April and were the result of pulse flow releases from La Grange Dam, mean fish size and age at exit for both the Tuolumne River (at La Grange and Modesto) and the Delta (at Vernalis) were generally similar to those of the above normal/wet WY types 1998 and 1999. However, confirmation of any relationship between mean fish size and age at exit and below normal/dry WY hydrology should consider the relatively small sample size (n=31) for these WY types and for outmigration year 2009 in particular (n=5).

5.3.3 Relationships between monthly flows and early life-history emigration patterns

Examination of mean monthly discharge, minimum monthly discharge, and maximum monthly discharge in the Tuolumne River at La Grange and Modesto for January through April did not reveal a discernable relationship with respect to growth rate, size at outmigration, or age at either outmigration or ocean entry for juveniles that originated in and returned to the Tuolumne River during the five years included in this study. Delta hydrologic patterns (at Vernalis) on a monthly timescale also did not exhibit clear relationships with growth rate, fish size, or age at ocean entry. Linear regressions indicated a lack of any compelling relationship ($R^2<0.4$, $p>0.1$) for the 192 combinations of fish size, fish age, monthly average flows for each of four months (January, February, March, April), and each of the 24 DAYFLOW parameters/indices (see Section 4.3).

6.0 DISCUSSION AND FINDINGS

Results of the analyses described above met both of the study objectives of using otolith microstructural growth patterns and/or microchemistry in order to identify:

- whether returning adults originated from hatcheries or riverine environments other than the Tuolumne River; and,
- growth rates and sizes of 'wild' fish at exit from the Tuolumne River and from the freshwater Delta.

These are discussed further below.

6.1 Hatchery origin fish

To provide an estimate of total hatchery contributions to Tuolumne River spawning escapement for the years examined in this study, the existing proportions of adipose fin clipped (i.e., hatchery marked) fish from CDFW annual spawning surveys can be combined with the proportions of unmarked hatchery fish estimated through otolith analysis. For each of the five outmigration years included in this study, a significant number of unmarked fish were classified as hatchery-origin fish through microstructural examination of otolith samples. The proportion of returning unmarked adults that originated in Central Valley hatcheries was greatest for the two below normal WY types (2003, 2009), exceeding the contribution from wild fish by approximately 2−4 times (Figure 5.1-1). The proportion of hatchery fish was relatively lower for above normal/wet WY types (1998, 1999, 2000), with the lowest proportion (33−44%) corresponding to outmigration year 1998 (Table 5.1-1). While these patterns are suggestive of a positive relationship between flow and the successful emigration of wild fish that later return as adults, confirmation of this relationship based on WY type should consider the relatively small sample size for below normal/dry WY types (n=31) vs. above normal/wet WY types (n=238).

Table 6.1-1 shows the proportions of marked (ad-clipped) and unmarked fish identified in the eight CDFW spawner survey years that recovered fish from outmigration years 1998, 1999, 2000, 2003, and 2009. The proportion of marked hatchery fish ranged from a low of 1% in 2006 to a high of 55% in 2011. For the unmarked fish, approximately 43% were identified as hatchery-origin (n=255) using results of the otolith analysis (Section 5.1). Combining the outmigration year unmarked hatchery contribution estimates with the known marked fish from subsequent escapement year surveys, Table 6.1-1 shows the total estimated hatchery contribution ranged from 39 to 100%, with a mean of 67% and generally increasing hatchery contribution in later years. To further refine this estimate and recognizing that some years in the otolith sample inventory over- and under-represent the typical age class structure in the escapement record, the overall proportion using only 3-year old recoveries, which are expected to make up the bulk of the annual escapement, ranges from 36 to 90%, with a mean of 58% (Table 6.1-1). Further consideration of large coded wire tag (CWT) releases to the Tuolumne River up to April 2005 suggests that some of the marked fish returning to the river during this period could be from the CWT release groups and thus would not be considered a true hatchery stray. Separating the Tuolumne River CWT release groups from all marked (ad-clipped) fish identified in the annual spawner surveys would reduce the estimated hatchery fractions for these years in Table 6.1-1. At the same time, large hatchery releases into the Tuolumne River may have swamped the existing predator population and increased outmigrant survival of emigrating wild fish. This would have the effect of slightly increasing the number of wild fish successfully emigrating and eventually returning to spawn. Nevertheless, it is apparent that hatchery contributions make up a large proportion of the annual spawning runs and the proportions of hatchery fish have been increasing in recent years.

Table 6.1-1. Estimated total hatchery contribution to annual escapement for spawner years corresponding to the five outmigration years included in the otolith study.

Spawner Year	CDFW spawner surveys			Including unmarked hatchery fish (all otolith samples)			Including unmarked hatchery fish (Age-3 otolith samples only)		
	Escapement[1]	Fraction Marked[2]	Marked Fish[2]	Unmarked Hatchery	Total Hatchery	Fraction Hatchery	Unmarked Hatchery	Total Hatchery	Fraction Hatchery
2000	17,873	6%	1,157	5,742	6,899	39%	5,207	6,364	36%
2001	9,222	16%	1,464	2,466	3,930	43%	2,667	4,131	45%
2002	7,125	31%	2,175	1,824	3,999	56%	1,566	3,742	53%
2005	719	11%	82	396	477	66%	396	477	66%
2006	625	1%	7	481	488	78%	-	-	-
2010	766	32%	245	521	766	100%	-	-	-
2011	2,847	55%	1,566	982	2,548	90%	982	2,548	90%
2012	2,120	29%	615	753	1,367	65%	-	-	-
					Mean	67%		Mean	58%

[1] Data source: Stillwater Sciences (2013c).
[2] Data sources: Annual CDFW spawning survey reports (e.g., CDFG 2010) and annual FishBio weir monitoring reports (e.g., Wright et al. 2013).

Overall, results of this study are consistent with observations of increasing hatchery contributions to salmon escapement in the Central Valley as a whole (Barnett-Johnson 2007, Johnson et al. 2011). The high proportions of marked and unmarked hatchery-origin fish represented in spawning runs to the Tuolumne River suggests that the influence of Project related effects as well as the ability to discriminate the effectiveness of potential measures intended to benefit Chinook salmon may be obscured by variations in the production and ocean survival of hatchery fish from the Merced River Fish Facility and other Central Valley hatcheries.

6.2 Growth and residence in the Tuolumne River and the Delta

Based on Sr isotope ratios ($^{87}Sr/^{86}Sr$) and otolith microstructural features, the study results suggest that mean fish size at exit from the Tuolumne River showed no apparent relationship with WY type, with the exception of outmigration year 2000 when mean fish size was significantly different ($p<0.005$) from the other four years of the study. Mean fish size at freshwater exit from the Delta also did not exhibit a relationship with WY type.

Age distributions at exit from the Tuolumne River and at exit from the Delta suggest that overall the total days of development from formation of otolith core to ocean entry for juvenile salmonids was relatively constant at 99±20 days for each of the five outmigration years included in the study. Fewer days spent rearing in the Tuolumne River resulted in relatively more days rearing in the Delta (Figure 5.2-1). The latter suggests extended rearing in the Delta for some parr-sized fish that emigrate early from the Tuolumne River. This is particularly evident in the average number of days spent in the Delta (27.6±12.1 days; Table 5.2-1) for outmigrating juveniles in 2000, which exceeded a more typical migration time of 14–21 days and suggests that some fish spent over 4 weeks in the Delta during the 2000 outmigration.

The particularly high parr (70%) representation in returning adults for outmigration year 2000 is interesting, especially given that year 2000 exhibited lower and later-peaking average daily flows than the other two above normal/wet years included in the study (1998, 1999). Consideration of spawner run timing in 1997, 1998, and 1999, which corresponds to outmigration years 1998, 1999, and 2000, suggests that the peak of spawning occurred 7–9 days earlier in 1997 and 1998 than the 1999 run corresponding to the year 2000 outmigration (Figure 6.2-1). The combination of earlier spawning during 1997 and 1998 and the extended high flows that occurred during 1998 and 1999 (Figure 5.3-1 and Figure 5.3-2) may have resulted in extended rearing and relatively higher numbers of fish emigrating at larger (i.e., smolt) sizes in these years than occurred in 2000. The peak of spawner run timing for the two below normal/dry WY types represented (i.e., spawner years 2002 and 2008) differ by only 3-days (Figure 6.2-1) and evaluation of any spawner timing issues related to below normal/dry WY types should consider the relatively small sample size (n=31) vs. above normal/wet WY types (n=238).

In summary, based upon the limited number of otoliths available for analysis by this study, it is apparent that early emigrating fish are not represented in subsequent spawning populations, with zero contributions in three out of five outmigration years analyzed and a maximum contribution of 5% in WY 2000. Consistent with observations of other tributary populations in the San Joaquin River basin, Tuolumne River parr and smolt outmigrants represented the vast majority of returning adults, implying a survival advantage for fish emigrating at larger sizes. The low fry contributions identified in this study suggest that any flow-related increases in the number of juvenile Chinook salmon leaving the Tuolumne River as fry may not necessarily result in corresponding increases in subsequent escapement. Additional analysis of adult otoliths from individuals emigrating in below normal/dry WY types in the future may help better discern whether below normal/dry runoff is associated with greater or lower representation of the size classes examined in this study.

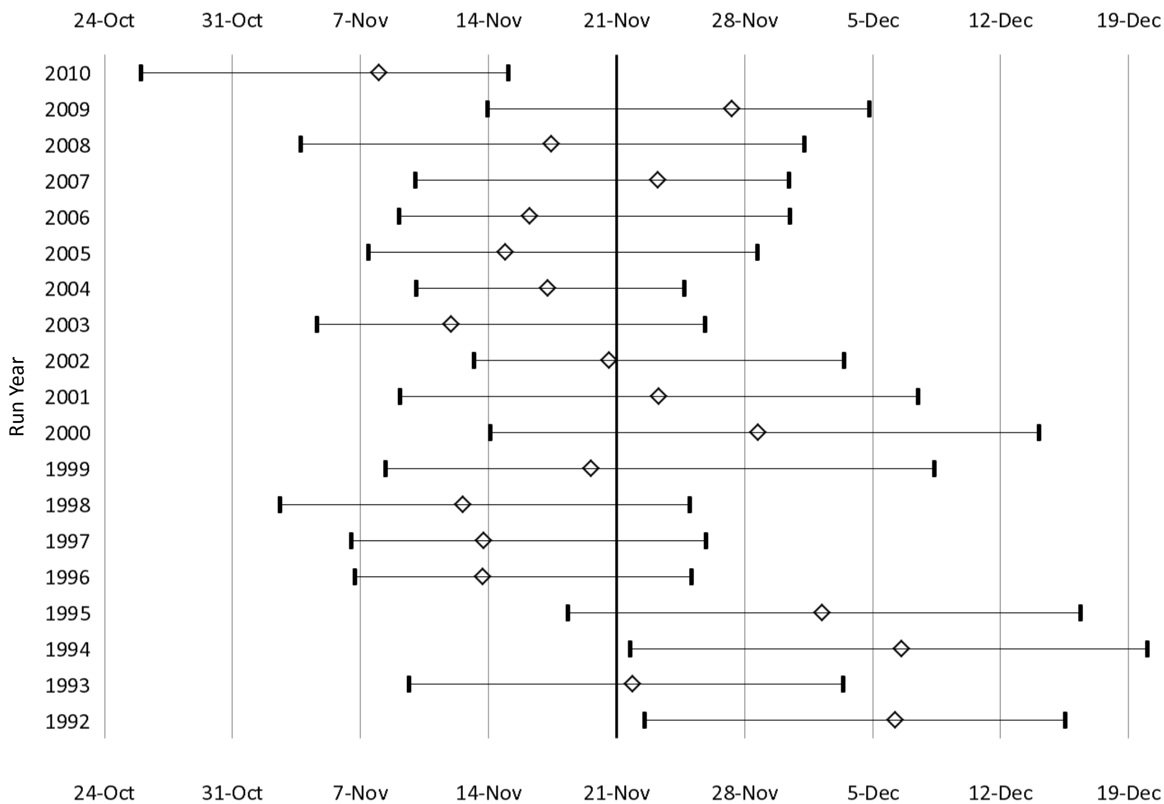

Figure 6.2-1. Tuolumne River spawner run-timing. Data sources: Annual CDFW spawning survey reports (e.g., CDFG 2010) and annual FishBio weir monitoring reports (e.g., Wright et al. 2013).

7.0 STUDY VARIANCES AND MODIFICATIONS

The study was conducted in conformance to the FERC-approved *Chinook Salmon Otolith Study Plan* (W&AR-11) approved in FERC's December 22, 2011 Determination. There are no variances.

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From: Staples, Rose
BCC To: Don Pedro Relicensing Participants Email Group
Sent: Monday, March 16, 2015 7:45 PM
Cc: Staples, Rose <Rose.Staples@hdrinc.com>
Subject: Attachment A to Don Pedro Otolith Study Report Forwarded Earlier Today

In the Don Pedro Chinook Salmon Otolith Study Report forwarded to you earlier today for your review and comment, there is reference to *Attachment A Tuolumne River Chinook Salmon Otolith Study – Analysis of Archival Otoliths Using Stable Isotope Microchemistry*. This attachment was not with the original report, so I am attaching it now. Both documents will also be uploaded shortly to the Don Pedro relicensing website at www.donpedro-relicensing.com. Thank you.

Rose Staples, CAP-OM
Executive Assistant

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TUOLUMNE RIVER CHINOOK SALMON OTOLITH STUDY - ANALYSIS OF ARCHIVAL OTOLITHS USING STABLE ISOTOPE MICROCHEMISTRY

Prepared by Drs. Anna Sturrock and Rachel Johnson as part of Don Pedro Project Relicensing (FERC No. 2299)

UNIVERSITY OF CALIFORNIA DAVIS

PERIOD 11/13-6/14

EXECUTIVE SUMMARY

Processes occurring in freshwater, estuarine, and marine habitats strongly influence the growth, survival and reproductive success of salmonids. One of the fundamental challenges in understanding salmon population dynamics lies in our inability to link and evaluate the relative importance of processes occurring throughout the complex salmon life cycle. For example, a critical unknown is the extent to which environmental conditions and management actions in the freshwater contribute to the expression and survivorship of different juvenile outmigration strategies into adulthood.

Here, we use Sr isotope ratios ($^{87}Sr/^{86}Sr$) and daily growth information recorded in Central Valley fall-run Chinook salmon, *Oncorhynchus tshawytcha,* otoliths ("earbones") to reconstruct the stream or hatchery-of-origin and early life movements of adult salmon collected on the Tuolumne River in the San Joaquin River Basin, California. A total of 598 paired otolith and scale samples were used to reconstruct and compare size-specific outmigration patterns for fish emigrating from the Tuolumne River in the spring of 1998, 1999, 2000, 2003 and 2009, incorporating dry, below normal, above normal and wet water year types. First, we identified adults that originated from the Tuolumne River (i.e. removed strays) using an updated 'strontium isoscape' and otolith growth characteristics exhibited by hatchery and wild salmon in the Central Valley [1, 2]. For each individual, otolith isotopic and microstructural data were linked with otolith radius in order to reconstruct the size and age at which they had exited from their natal river and from freshwater. Back-calculated fork lengths (± 95% CI) were used to classify outmigrants into one of three life history stages: fry (≤55mm), parr (>55mm to ≤75mm) or smolt (>75 mm).

Our study shows that a significant number of adults spawning in the Tuolumne River in fall of 2000-2012 were strays from other rivers and hatcheries in the Central Valley. The earliest three outmigration years examined had relatively low straying rates of unmarked fish, with a greater proportion of spawners having originated in and reared in the Tuolumne River (1998: 57-68% returns, 33-44% strays; 1999: 38-53% returns, 47-62% strays; 2000: 61-64% returns, 36-39% strays). Outmigration year 2003 exhibited an intermediary straying rate (27-35% returns, 65-73% strays) while outmigration year 2009 was subject to particularly high straying rates (9-15% returns, 85-91% strays, primarily from the Coleman National Fish Hatchery on Battle Creek in the Sacramento River watershed, which comprised 57% of the unmarked sample).

All size classes of juvenile outmigrants were represented in the adult spawning populations. Tuolumne-origin adults were largely comprised of individuals that had emigrated from the Tuolumne River as parr and smolts, however, in outmigration year 2000, 20% of the returning adults had outmigrated as fry. Comparable with findings on other rivers in the San Joaquin Basin, parr outmigrants were consistently the most commonly observed phenotype in the returning adults.

INTRODUCTION

Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exhibit significant variation in the size, timing and age that they emigrate from their natal rivers [14]. Typically, juveniles rear in the freshwater for one to three months before smoltification prompts downstream migration towards the ocean; however, early spring flows are often also coupled with large pulses of emigrating fry [5, 14, 17]. In some years, fry-sized individuals are the most numerous size-class leaving natal rivers and entering the delta [17, 18]. The contribution of these smaller outmigrants to the adult population is often assumed to be negligible, as juvenile survival is generally positively correlated with body size [e.g. 19] and there is little evidence for significant downstream rearing in the San Francisco estuary [20]. Hatcheries tend to release larger smolts to maximize survival rates and their contribution to the ocean fishery, but a recent study indicated that the majority of California Central Valley (CCV) adults captured in the Oregon troll fishery had emigrated as fry and parr [21]. Scale analyses have also inferred greater survival rates of intermediate-sized juveniles [22]. Understanding the relative survivorship of different outmigrant size classes is critical to our understanding of population dynamics and evaluation of freshwater management actions and water operations.

Quantifying the relative contribution of different size classes and/or developmental stages of juvenile salmon to the adult spawning population has largely been limited by the methodological challenges associated with reconstructing early life history movements of the adults. Mark-recapture studies using coded wire tags (CWT) have provided empirical indices of juvenile survival rates through the Sacramento-San Joaquin system [28], but are hindered by low rates of return and often use hatchery fish, which may exhibit different behavior and survival than their wild counterparts [29]. No study to date has tracked habitat use of individual salmon over an entire lifecycle to estimate the relative success or survivorship of juvenile outmigration phenotypes, let alone under different flow conditions or between different rivers in the same year. Most have relied on correlations between environmental conditions (e.g. flow) experienced during juvenile outmigration periods and abundance of returns [16, 30].

Recent advances in techniques using chemical markers recorded in biomineralized tissues provide rare opportunity to retrospectively "geolocate" individual fish in time and space [31]. Otoliths are metabolically inert, calcium carbonate "earbones" found in all bony fishes, that grow incrementally from birth (the otolith "primordia") to death (the outer edge of the otolith). The otolith microstructure features daily and annual growth rings that can be determined visually using light microscopy [32]. In Chinook salmon, as the otoliths grow proportionally to fish length during juvenile stages, daily increment widths can be used to reconstruct individual growth trajectories, providing a means to compare growth rates across life stages, hydrologic regimes and contrasting environments. Otolith microstructure can therefore provide insights into how juvenile salmon growth is affected by biotic and abiotic factors such as food availability and water temperature. When microstructural and microchemical techniques are combined, otoliths can provide a powerful natural tag for reconstructing movement patterns of individual fish [33]. The technique relies on differences in the physicochemical environment producing a distinct and reproducible "chemical fingerprint" in the otolith. In the CCV, strontium isotopes ($^{87}Sr/^{86}Sr$) are ideal markers because the water signature varies with the parent geology, differing among many of the rivers and salmon outmigration paths, and is faithfully recorded in the otoliths of Chinook salmon [1, 34]. Changes in otolith $^{87}Sr/^{86}Sr$ values can be used to reconstruct time- and age-resolved movements as salmon migrate through the freshwater, estuarine, and ocean environments [1, 34]. Furthermore, in salmon, otolith size is significantly related to body size [32, 35, 36], allowing back-calculation of individual fork length (FL) at specific life history events.

Here, we used otolith $^{87}Sr/^{86}Sr$ ratios and microstructure to identify natal origin and reconstruct size/age at emigration of adults that spawned in the Tuolumne River in 1996-2008. These adults represent cohorts that emigrated as juveniles from the freshwater in 1998, 1999, 2000, 2003 or 2009. First we used the otolith data to differentiate between adults that strayed from other rivers from adults that were born and returned to the Tuolumne River. After removing strays from other rivers, we used otolith $^{87}Sr/^{86}Sr$ ratios, growth increments and radii to determine the size and age at which returning (i.e. "successful") adults had originally emigrated from the Tuolumne River and from the freshwater system. We aimed to address the following questions:

1. What was the early fresh-water life history of the adult Chinook salmon? More specifically, at what age (days from exogenous feeding) and estimated size did the returning adult leave the Tuolumne River as a juvenile?
2. What was the origin of the adult Chinook salmon? More specifically, what portion of the adult Chinook salmon escaping to the Tuolumne River originated from the Tuolumne River separate from hatcheries and other riverine environments of the Sacramento and San Joaquin Central Valley drainages?

STUDY AREA

The Tuolumne River is one of the southernmost tributaries of the San Joaquin River (SJR) (Fig. 1). The lower basin typically experiences a Mediterranean climate with wet winters and dry summers, and the tributaries are predominantly fed by snowmelt from the Sierra Nevada Mountains. The Tuolumne watershed encompasses a 1,900 square-mile area of the central Sierra Nevada and northern San Joaquin Valley and includes the northern half of Yosemite National Park. The Tuolumne is the largest tributary to the SJR, producing an average annual unimpaired yield of 1,906,000 acre-feet. The river flows for 150 miles from its headwaters at over 13,000 ft on Mt. Dana and Mt. Lyell to its confluence with the SJR at an elevation of 30 ft. The lower Tuolumne extends from its confluence with the SJR to La Grange Dam at river mile (rm) 52.2, which has been the upstream barrier to anadromous fish movements since at least 1871 [10].

Around 90% of the annual precipitation on the Tuolumne River occurs between November and April, with an annual minimum flow schedule including migration pulse flows in April and May required by the Federal Energy Regulatory Commission (FERC 1996).

METHODS

ADULT SAMPLING AND COHORT RECONSTRUCTION

Otoliths were extracted from age 2, 3 and 4 year old adults in the Tuolumne River during carcass surveys conducted by CDFW in the fall of 2000-2012 (Table 1). The five focus years of the current study (1998, 1999, 2000, 2003 and 2009) encompassed a range of hydrologic conditions (wet, above normal, above normal, below normal and dry, respectively) based on the San Joaquin valley water index (http://cdec.water.ca.gov). Carcass surveys were typically run from October to early-January depending on abundance and hydrologic conditions. Sample selection was temporally stratified to follow the same cohort across different escapement years, as fish return at different ages. This approach was taken to capture the age structure typically observed for salmon in the San Joaquin tributaries. This was deemed important in order to capture a representative sample that accounted for the potential for the outmigration strategy to co-vary with age-at-return. For example, it is unclear the extent to which larger outmigrants may have a higher likelihood of returning as younger (age 2) adults. Our sampling design was not intended to explicitly test whether there was a linkage between outmigration strategies and return age, however. Ages and outmigration cohorts were determined by counting scale winter annuli by experts at CDFW La Grange, as per established and validated techniques [41].

Otolith treatment and $^{87}Sr/^{86}Sr$ analyses

Otoliths were prepared and analyzed for $^{87}Sr/^{86}Sr$ ratios by multi-collector laser ablation inductively coupled plasma mass spectrometry (MC-LA-ICPMS) using the methods described in Barnett-Johnson et al. [2]. In brief, otoliths were rinsed 2-3 times with deionized water and cleaned of adhering tissue. Once dry, otoliths were stored in clean microcentrifuge tubes then mounted in Crystalbond™ resin and polished (600 grit, 1500 grit, 3 µm then 1 µm lapping film) until the primordia were exposed. $^{87}Sr/^{86}Sr$ analyses were carried out on a Nu plasma HR (Nu Instruments Inc.) interfaced with a Nd:YAG 213 nm laser (New Wave Research) at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry. Contrasting with the line transects used to establish natal signatures of tributaries in the CCV [1, 2] we used spot analyses to prevent cross-contamination of ablated material and to allow coupling of chemical data with discrete microstructural features. A 40µm or 55µm laser beam diameter was used (roughly equivalent to 10-14 days of growth) with pulse rate of 20 or 10 Hz at 70 or 65% power and a dwell time of 25 or 35 seconds. Helium was used as the carrier gas to improve sensitivity and was mixed with argon before reaching the plasma source. Gas blank and background signals were monitored following sample changes and measured for 30 seconds prior to each batch of spot analyses. A modern coral sample was analyzed at the start of each analytical session and the outer (marine) portion of adult salmon otoliths was analyzed between every otolith. The measured $^{87}Sr/^{86}Sr$ ratio was normalized to $^{86}Sr/^{88}Sr = 0.1194$ and to maximize accuracy, batches of unknowns were corrected to the global $^{86}Sr/^{88}Sr$ value (0.70918) by correcting to the mean of three spot analyses on the marine portion of an adult salmon otolith analyzed immediately afterwards.

A standardized 90° transect was used for $^{87}Sr/^{86}Sr$ and otolith radius measurements, starting at the post-rostrum primordia going in the dorsal direction (Fig. 2). Juvenile otoliths of known origin (from previous studies) were used to assign natal origins of adults in the current project. In the juvenile otoliths, the transect was terminated at the otolith edge to ensure analysis of the most recently deposited material in order to characterize capture site (natal) signature. In the adult otoliths of unknown origin, the transect was terminated past the ocean entry check or to a distance of c.800µm (c. 120mm FL) to ensure inclusion of the full freshwater outmigration period. To improve the spatial resolution and accuracy of exit spot identification and back-calculated FL, additional $^{87}Sr/^{86}Sr$ analyses were carried out around the Tuolumne-SJR transition. These additional spots ("respots") meant that generally, subweekly resolution could be achieved.

STRONTIUM ISOSCAPE

As part of ongoing work to provide better resolution on the determination of fish origin useful in this study, Sr isotope values of known-origin otolith samples from juveniles and CWT adults were combined with the previously published $^{87}Sr/^{86}Sr$ baseline [1]. Water samples (A. Sturrock, unpublished) were combined with data from Ingram and Weber (1999) and P. Weber (unpublished). The resulting 'strontium isoscape' was comprised of 480 samples from all potential natal sources in the CCV, with many sites sampled across multiple years (1998-2013) and hydrologic regimes (Table 3). Thus, the isoscape can be quantitatively characterized by the mean $^{87}Sr/^{86}Sr$ isotope values and the standard deviations for the different salmon rivers and hatcheries in the CCV.

Otoliths from juveniles collected from their natal tributary or hatchery were analyzed for $^{87}Sr/^{86}Sr$ using the same type of transect as the adults, and the natal signature determined from otolith material deposited immediately after onset of exogenous feeding (~250μm from the core, see [2]). Material deposited prior to this point exhibits an elevated signature due to the influence of maternally-derived strontium from the yolk, which for fall-run salmon, was formed while the mother was in the ocean.

IDENTIFICATION OF NATAL ORIGIN

In order to reconstruct juvenile outmigration strategies for the Tuolumne River salmon population, it was critical to remove any fish that had strayed from other tributaries or hatcheries. Given that hatcheries tend to release at larger sizes [21], not detecting and removing hatchery strays in our analyses would likely bias the representation of smolt outmigrants. To identify the origin of our unknown fish, we measured the natal $^{87}Sr/^{86}Sr$ and then statistically determined which river or hatchery in the strontium isoscape (see previous section) had the most similar $^{87}Sr/^{86}Sr$ to the unknown fish. The utility of using a linear discriminant function analysis (DFA) to classify unknown origin fish into their likely rivers/hatcheries of origin, is that it allows one to use additional sources of information. In this case, we can use previous observations of hatchery strays from coded wire tag recoveries in the Constant Fractional Marking Report (probabilities/group weightings) and use that information to help weight our statistical model to more accurately account for hatchery strays (Table 2) [42, 43]. Thus, the DFA approach allowed us to incorporate empirical data of stray-rates from the major hatcheries into our statistical model to account for non-random patterns in salmon straying and improve classification accuracy. As the majority of Chinook salmon return to freshwater at 3 years old [14], the more recent report (escapement year 2011 [42]) was cohort-matched to outmigration year 2009 (escapement year – outmigration year + 1). All adults from previous outmigration cohorts were assigned using priors from the earlier CFM report [43].

The natal signature was determined by averaging the $^{87}Sr/^{86}Sr$ values that corresponded with the otolith material deposited immediately after onset of exogenous feeding (but prior to emigration from the natal river). The DFA assignments for the mean natal value were used to determine the river or hatchery of origin. Juveniles collected in the Tuolumne River exhibit more variable isotopic signatures within and among individuals than in other rivers in the CCV (see Results). Some juveniles that were collected in the Tuolumne River exhibited $^{87}Sr/^{86}Sr$ values that appeared to imply movement into the SJR or Stanislaus River immediately after emergence and then return to the Tuolumne (e.g. Fig. 3C). However, given that the changes in isotopic values tended to occur at early stages, when individuals are unlikely to be strong enough swimmers to move freely up and downstream, we interpreted this pattern to represent geographic variations in the $^{87}Sr/^{86}Sr$ signature within the Tuolumne River, confirmed with additional water sampling carried out as part of other projects (Fig. 1 & 8).

As the Tuolumne River exhibits variable water chemistry from upper to lower reaches (P. Weber, A. Sturrock, unpublished), and otolith $^{87}Sr/^{86}Sr$ values of known-origin fish from the Tuolumne River, Mokelumne River Hatchery and Feather River Hatchery can overlap (see Results), there is a potential of misclassifying Tuolumne-origin fish. Thus, to improve our assignment accuracy, any individuals exhibiting ambiguity in their natal assignment were also analyzed for otolith microstructural features that can discriminate hatchery from wild fish. We used the methods developed for CCV Chinook [44], where individuals are classified as hatchery or wild based on the prominence of the exogenous feeding check (scored blind by 2-3 independent readers) and the mean and variance in increment width around the first 30 daily increments following onset of exogenous feeding.

RECONSTRUCTING SIZE AND AGE AT OUTMIGRATION

Emigration from the Tuolumne River ('natal exit') was determined using the distance from the core to the 'last natal spot' rather than the 'first non-natal spot', because to accrete sufficient new otolith material to modify the isotopic composition of the otolith, the fish would have inhabited isotopically distinct (i.e. non-natal) water for several days, after which time it would be a significant distance downstream of the confluence. The method used to identify the 'last natal spot' was to work backwards from the final inflection point indicative of ocean-bound migration (Fig. 3A-C). We assumed that the lowest point of this final inflection represented movement through the SJR, and thus used the spot prior as the last natal spot. The only exceptions were on occasions when the lowest point prior to ocean migration was lower than any value measured in the SJR (e.g. Fig. 3D); on these occasions the lowest point was assumed to have been deposited while the fish was rearing in the lower Tuolumne River, which has been shown to exhibit values as low as 0.7066 (P. Weber, A. Sturrock, unpublished). Emigration from freshwater ('freshwater exit') was defined as the distance at which otolith $^{87}Sr/^{86}Sr$ values last reached 0.7080 (equivalent to 1ppt based on [45]), determined using linear interpolation.

To back-calculate fish size at natal and freshwater exit, the relationship between otolith radius and FL was quantified using fall run Chinook salmon juveniles from the same "Evolutionarily Significant Unit" (ESU), which is of utmost importance for producing relevant and unbiased back calculation models [46]. Otolith radius was measured using a Leica DM1000 microscope and Image Pro Plus 7. Reference samples were collected as part of other projects from the Tuolumne River (2003; n = 6), Stanislaus River (2000 and 2002; n = 95), the Coleman National Fish Hatchery (2002; n=40) and in the San Francisco Bay at Golden Gate Bridge (2005; n = 83) (Fig. 5). The Tuolumne-origin fish tended to sit above the mean regression line (Fig. 5), but there was no significant difference between the back-calculated FL of Tuolumne vs. non-Tuolumne fish (ANCOVA: $p = 0.08$), nor any difference in the slopes (ANCOVA: $p = 0.8$). As such, we assumed that the overall OR-FL relationship was suitable for reconstructing FLs of juveniles from the Tuolumne River, however it would be advisable to increase representation of Tuolumne-origin juveniles in future analyses. The error around the OR-FL calibration line (Fig. 5) was used to estimate 95% confidence intervals (CI) around individual FL reconstructions. Individuals were categorized as fry, parr or smolt outmigrants based on FL: ≤55mm, >55 to <75mm, and >75mm FL, respectively (after [21]).

Daily growth bands were counted and widths between daily increments were measured along the same 90 degree transect as the geochemical analysis, beginning at the post exogenous feeding check until freshwater exit. Some otoliths were difficult to age and given low readability scores (1-2); ages are not provided for these individuals. The ages of fish at Tuolumne River exit, Freshwater exit, and habitat-specific growth rates were obtained for fish with otolith readability scores of 3-5. A subset of otoliths were aged by two independent readers, providing an estimate of error associated with fish aging. The two independent reads of each fish demonstrated high agreement, with an average difference of ± 5 days (range 0-12 days).

RESULTS

ACCURACY OF NATAL ASSIGNMENTS

The DFA assigned 63% of samples back to the correct site of origin (Table 4), with the majority of misclassified sites being among the Mokelumne River Hatchery (MOH), Feather River Hatchery (FEH) and the Tuolumne River (TUO), which overlap in their chemical composition (Fig. 6). The use of otolith microstructure (~10% error rate for hatchery vs. wild assignments) [44] and weighted priors helped to separate TUO-origin fish from MOH and FEH strays, however there remains potential for misclassifications between the two hatchery sites (FEH and MOH), particularly given that (except for outmigration year 2009) the priors used were not cohort-specific. We prepared and processed 13 CWT fish from outmigration years 1999 and 2000 of known hatchery origin. However, the presence of these samples was withheld from the individuals preparing the samples, collecting the $^{87}Sr/^{86}Sr$ data, as well as statistically assigning them to natal origin. Thus, these known samples were treated in the same way as all the unknowns in the study. Once the assignments were made, the true identify of these fish were revealed to the analysts. All fish were correctly classified to the Merced River Hatchery (MEH).

PATTERNS IN $^{87}SR/^{86}SR$ VALUES WITHIN THE TUOLUMNE RIVER

Contrary to the stable $^{87}Sr/^{86}Sr$ profiles observed in other CCV rivers, the Tuolumne River is characterized by variable $^{87}Sr/^{86}Sr$ values from the upper spawning reaches to the confluence with the San Joaquin River (A. Sturrock, unpublished). This variability was first observed in some water analyses (P. Weber, unpublished) and known-origin juveniles (Fig. 3C & D), and subsequently in adult otolith $^{87}Sr/^{86}Sr$ profiles from outmigration years 2000 and 2003 [47]. The lower isotopic values in the lower river were originally hypothesized to result from inputs of Stanislaus River water via Dry Creek (a tributary to the Tuolumne River at river mile [rm] 17). However, subsequent water analyses (carried out as part of other studies) indicated declines in $^{87}Sr/^{86}Sr$ values as far upstream as rm46, with rm 22 to the confluence exhibiting relatively stable signatures around 0.7065 (Fig. 8). The average variability (2SD) of the water analyses based on analyses of multiple standard reference materials was 0.000020, providing high confidence in these data. The geographic trends in Tuolumne River water $^{87}Sr/^{86}Sr$ cannot be explained by inputs from Dry Creek alone (rm 17), implying additional sources of isotopically light water to the upper and mid reaches of the river.

These patterns have clear implications for identifying fish origin, determining rearing location(s) within the Tuolumne River, and the rules used to identify transitions between the Tuolumne and San Joaquin rivers (Fig. 2, 3). Trace elemental analyses of water samples carried out as part of past and ongoing projects (P. Weber, A. Sturrock, unpublished) indicate clear differences in water Sr/Ca and Ba/Ca ratios between the Tuolumne and San Joaquin Rivers (Fig. 9). Thus, future studies attempting to identify fish transition across this confluence might benefit from a multi-elemental approach, combining otolith Sr isotopes with Sr/Ca and Ba/Ca analyses [48].

Straying and return rates to the Tuolumne River

Overall, straying rates of unmarked fish have increased over time coincident with increasingly dry environmental conditions. The earliest three outmigration years examined had relative low straying rates of unmarked fish (1998: 57-68% returns, 33-44% strays, 1999: 38-53% returns, 47-62% strays, 2000: 61-64% returns, 36-39% strays). Outmigration year 2003 had intermediary straying rates (27-35% returns, 65-73% strays), while outmigration year 2009 was characterized by particularly high straying rates (9-15% returns, 85-91% strays, primarily from the Coleman National Fish Hatchery on Battle Creek, which comprised 57% of the total sample).

Size and age at outmigration

Given the variance around the mean OR-FL regression line (approximately ±10mm FL; Fig. 5), it is not advisable to place too much emphasis on any one particular FL reconstruction; with the upper and lower FL estimates often resulting in fish spanning multiple life stages (Appendix 1A & B). However, given a lack of bias in the OR-FL relationship, and its consistency between Sacramento and San Joaquin basin-origin fish (Fig. 5), the average FLs and overall life stage assignments (Tables 6 and 7) were deemed relatively robust and representative population-level metrics.

All size classes of juvenile outmigrants were represented in the adult spawning population. Tuolumne-origin adults were largely comprised of individuals that had emigrated from the Tuolumne as parr and smolts, however, in outmigration year 2000, 20% of the returning adults had outmigrated as fry (Table 6). Consistent with observations of other populations in the San Joaquin Basin, parr outmigrants were generally the most commonly observed phenotype in the returning adults, implying a potential survival advantage despite being smaller than smolts. There were significant differences in size, age and growth rate between outmigration years ($p<0.05$, Fig. 7, Table 9), but no inter-annual difference in growth rate variability (as tested through comparisons of the coefficient of variation in increment width; $p>0.05$). In general, outmigration year 2000 was characterized by younger, smaller outmigrants; however, the number of days in the freshwater delta was longer (Fig. 9), implying a higher frequency of non-natal rearing during this season.

TABLES

Table 1. Numbers of otolith samples sampled randomly from unclipped salmon carcasses in the Tuolumne River between 2000 and 2012. Ages were obtained from CDFW scale readings and samples matched to outmigration years 1998, 1999, 2000, 2003 and 2009 before Sr isotope analysis.

Cohort		**Adult carcass sampling year**	**Age at return (yr)**	**Number of individuals**	**% of total sample**
Brood year	**Outmigration year (WYT†)**				
1997	1998 (Wet)	2000	3	124	62%
		2001	4	76	38%
1998	1999 (Above normal)	2000	2	9	6%
		2001	3	64	44%
		2002	4	73	50%
1999	2000 (Above normal)	2001	2	31	28%
		2002	3	79	72%
		2003	4	0	0%
2002	2003 (Below normal)	2004	2	0	0%
		2005	3	87	91%
		2006	4	9	9%
2008	2009 (Dry)	2010	2	14	30%
		2011	3	30	65%
		2012	4	2	4%
TOTAL				598	

† San Joaquin Valley Index Water year type during juvenile rearing & outmigration

Table 2. Discriminant Function Analysis (DFA) priors used in the current study to predict natal origin of adults obtained in the Tuolumne River Carcass Survey corresponding to outmigration years 1998, 1999, 2000, 2003 and 2009. The probabilities are based on the CWT-derived proportions of hatchery strays in the Tuolumne in escapement year 2010 and 2011 constant fractional marking (CFM) reports and an assumed natural straying rate of 5% [49], removed from the proportion of "natural" fish reported in the CFM report and divided equally among the remaining salmon rivers in the California Central Valley. Priors from CFM escapement year 2010 were applied to all cohorts pre-2009, while priors from CFM escapement year 2011 were applied to outmigration year 2009, given cohort-matching to the dominant year class. Note that Feather River Hatchery and Thermalito Rearing Annex were not distinguished between in the CFM reports, so the priors for the former were divided equally between the two sites.

Natal origin	**Site code**	**"Wild" or hatchery**	**Prior probability based on CFM 2010 escapement** *(all outmigration years <2009)*	**Prior probability based on CFM 2011 escapement** *(outmigration year 2009 only)*
Tuolumne River (RETURNS)	TUO	W	0.4845	0.2565
Merced River Hatchery	MEH	H	0.1060	0.2081
Feather River Hatchery	FEH	H	0.0624	0.0684
Thermalito Rearing Annex	THE	H	0.0624	0.0684
Nimbus Hatchery	NIM	H	0.0433	0.0116
Coleman National Fish Hatchery	CNH	H	0.1345	0.0848
Mokelumne River Hatchery	MOH	H	0.0569	0.2524
Battle Creek	BAT	W	0.005	0.005
Deer Creek	DEE	W	0.005	0.005
Mill Creek	MIL	W	0.005	0.005
Butte Creek	BUT	W	0.005	0.005
Feather River	FEA	W	0.005	0.005
Stanislaus River	STA	W	0.005	0.005
Mokelumne River	MOK	W	0.005	0.005
Yuba River	YUB	W	0.005	0.005
Merced River	MER	W	0.005	0.005
American River	AME	W	0.005	0.005

Table 3. Details of samples and mean $^{87}Sr/^{86}Sr$ included in the DFA to assign natal origin (n=480), where "matrix" includes juvenile otoliths (J), CWT adult otoliths (CWT) and water samples (W). All analyses were carried out as part of existing or ongoing projects ([1], [34], P. Weber, A. Sturrock, unpublished), and used to predict the origin of adults collected in the current study. Site codes are provided in Table 2.

Site	Matrix	Year	N	Mean 87Sr/86Sr	SD
AME	J	1999	5	0.71025	0.00004
AME	W	1998	4	0.70979	0.00017
BAT	J	1999	9	0.70391	0.00017
BUT	W	1998	5	0.70481	0.00009
CNH	CWT	2000	1	0.70527	
CNH	CWT	2009	7	0.70547	0.00043
CNH	CWT	2010	3	0.70557	0.00013
CNH	J	2000	5	0.70531	0.00020
CNH	J	2002	8	0.70535	0.00038
DEE	J	2002	8	0.70412	0.00004
DEE	W	1998	5	0.70409	0.00003
FEA	J	1999	5	0.70622	0.00012
FEA	J	2000	5	0.70621	0.00020
FEA	J	2002	8	0.70615	0.00003
FEA	W	1998	7	0.70620	0.00011
FEH	CWT	2007	14	0.70728	0.00013
FEH	CWT	2008	19	0.70741	0.00014
FEH	J	1999	5	0.70673	0.00012
FEH	J	2000	5	0.70736	0.00017
FEH	J	2002	17	0.70717	0.00020
FEH	J	2004	5	0.70709	0.00014
MEH	CWT	1998	5	0.70888	0.00009
MEH	CWT	1999	5	0.70886	0.00006
MEH	CWT	2001	6	0.70854	0.00006
MEH	CWT	2003	6	0.70872	0.00006
MEH	CWT	2004	2	0.70862	0.00004
MEH	CWT	2006	5	0.70892	0.00007
MEH	CWT	2009	6	0.70871	0.00002
MEH	CWT	2010	6	0.70865	0.00010
MEH	J	1999	1	0.70885	
MEH	J	2002	9	0.70861	0.00003
MEH	J	2004	5	0.70869	0.00011
MER	J	2003	13	0.70852	0.00010
MER	W	1998	4	0.70846	0.00063
MIL	J	2002	10	0.70412	0.00003
MIL	W	1998	5	0.70396	0.00002
MOH	CWT	1998	2	0.70742	0.00003
MOH	CWT	1999	6	0.70767	0.00011
MOH	CWT	2000	13	0.70757	0.00009
MOH	CWT	2001	7	0.70751	0.00009
MOH	CWT	2002	4	0.70757	0.00012
MOH	CWT	2007	8	0.70736	0.00010
MOH	CWT	2008	5	0.70744	0.00014
MOH	CWT	2009	6	0.70737	0.00009
MOH	CWT	2010	8	0.70723	0.00007
MOH	J	1999	4	0.70768	0.00008
MOH	J	2000	5	0.70760	0.00007
MOH	J	2002	11	0.70755	0.00013
MOK	J	2000	4	0.70709	0.00005
MOK	J	2002	10	0.70690	0.00004
MOK	W	1998	4	0.70696	0.00016
NIH	J	2002	9	0.70974	0.00006
STA	J	1999	7	0.70663	0.00002
STA	J	2000	7	0.70663	0.00004
STA	J	2002	10	0.70656	0.00011
STA	J	2011	3	0.70646	0.00005
STA	J	2012	12	0.70643	0.00007
STA	J	2013	7	0.70641	0.00011
STA	W	2012	5	0.70639	0.00002
THE	J	2004	5	0.70581	0.00011
TUO	J	1999	3	0.70783	0.00042
TUO	J	2003	6	0.70757	0.00022
TUO	J	2007	34	0.70763	0.00019
TUO	J	2010	7	0.70780	0.00014
TUO	J	2011	4	0.70780	0.00003
TUO	W	1998	5	0.70789	0.00025
TUO	W	2013	2	0.70785	0.00006
YUB	J	2002	19	0.70823	0.00021

Table 4. Performance of the unweighted DFA for natal assignments. For the unknown samples in this study, weighted priors were used (Table 2) and hatchery vs. wild assignments based on otolith microstructure improved classification accuracy [44].

Site	BAT	DEE	MIL	BUT	CNH	THE	FEA	STA	MOK	FEH	MOH	TUO	YUB	MER	MEH	NIH	AME	Total	% Correct
BAT	**7**	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	78%
DEE	0	**8**	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	62%
MIL	5	9	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	7%
BUT	0	0	0	**5**	0	0	0	0	0	0	0	0	0	0	0	0	0	5	100%
CNH	0	0	0	4	**14**	5	1	0	0	0	0	0	0	0	0	0	0	24	58%
THE	0	0	0	0	0	**5**	0	0	0	0	0	0	0	0	0	0	0	5	100%
FEA	0	0	0	0	0	1	**22**	2	0	0	0	0	0	0	0	0	0	25	88%
STA	0	0	0	0	0	0	4	**47**	0	0	0	0	0	0	0	0	0	51	92%
MOK	0	0	0	0	0	0	0	0	**15**	3	0	0	0	0	0	0	0	18	83%
FEH	0	0	0	0	0	0	0	2	13	**26**	24	0	0	0	0	0	0	65	40%
MOH	0	0	0	0	0	0	0	0	0	19	**35**	25	0	0	0	0	0	79	44%
TUO	0	0	0	0	0	0	0	0	1	2	18	**35**	5	0	0	0	0	61	57%
YUB	0	0	0	0	0	0	0	0	0	0	0	1	**14**	4	0	0	0	19	74%
MER	0	0	0	0	0	0	0	0	0	0	0	1	0	**12**	4	0	0	17	71%
MEH	0	0	0	0	0	0	0	0	0	0	0	0	0	14	**42**	0	0	56	75%
NIH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	0	9	100%
AME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	**6**	9	67%
OVERALL																			63%

Table 5. Natal origin of all unclipped fish analyzed for 5 outmigration years (1998, 1999, 2000, 2003 and 2009). Note that adclipped fish have been removed (1 from OMY 1999, 12 from OMY 2000 - all correctly assigned to Merced Hatchery).

	Site	Code	1998 N	1998 %	1999 N	1999 %	2000 N	2000 %	2003 N	2003 %	2009 N	2009 %
Wild	Tuolumne R.	TUO	117	59%	55	38%	66	61%	26	27%	5	11%
	Tuolumne R.*	TUO*	17	9%	22	15%	2	2%	8	8%	2	4%
	Stanislaus R.	STA	5	3%	8	5%	4	4%	0	0%	0	0%
	Merced R.	MER	1	1%	0	0%	0	0%	0	0%	0	0%
	Mokelumne R.	MOK	2	1%	1	1%	0	0%	0	0%	0	0%
	Feather R.	FEA	0	0%	0	0%	0	0%	0	0%	1	2%
Hatchery	Coleman H.	CNH	0	0%	0	0%	0	0%	1	1%	26	57%
	Feather H.	FEH	1	1%	5	3%	4	4%	6	6%	4	9%
	Mokelumne H.	MOH	8	4%	28	19%	23	21%	39	41%	1	2%
	Merced H.	MEH	34	17%	9	6%	5	5%	4	4%	2	4%
	Nimbus H.	NIH	0	0%	5	3%	1	1%	9	9%	2	4%
	Thermalito (Feather H.)	THE	0	0%	2	1%	0	0%	3	3%	3	7%
	Habitat X ‡	X	15	8%	11	8%	5	5%	0	0%	0	0%
	Total		**200**		**146**		**110**		**96**		**46**	

* Individuals assigned to the Tuolumne with <0.5 posterior probability based on mean natal $^{87}Sr/^{86}Sr$ values.

‡ Individuals assigned as hatchery-origin based on otolith microstructure, but where natal $^{87}Sr/^{86}Sr$ values are outside of the observed range of any hatchery in the CCV.

Table 6. Life stage † at natal exit for fish assigned to the Tuolumne River with high confidence

Outmigration year	N	Fry	Parr	Smolt
1998	117	2%	56%	43%
1999	55	4%	62%	35%
2000	67	20%	73%	8%
2003	26	4%	65%	31%
2009	5	0%	40%	60%

† Life stage defined as fry (≤55mm), parr (>55mm to ≤75mm) or smolt (>75 mm) after [21]

Table 7. Summary of average forklength (FL) at exit, number of increments (days) and increment width (growth rate) in the natal river and freshwater delta by outmigration year for juveniles that originated in and returned to the Tuolumne River (identified as "TUO" in Appendix Table 1). Trends are also visualized in Figure 9 in the form of box plots (i.e. displaying median values as opposed to means), alongside the results of statistical comparisons among years.

		Natal river			Freshwater delta		
Outmigration year	Sample size	FL at exit (mm)	No. increments (days)	Increment width (µm)	FL at exit (mm)	No. increments (days)	Increment width (µm)
1998	117	73.3 ± 8.5	91.0 ± 16.2	3.07 ± 0.28	80.8 ± 9.0	15.8 ± 7.5	3.24 ± 0.54
1999	55	72.6 ± 11.6	82.0 ± 13.6	3.20 ± 0.27	82.3 ± 11.5	16.5 ± 8.7	3.35 ± 0.56
2000	66	63.5 ± 8.6	68.5 ± 18.6	3.10 ± 0.26	77.4 ± 6.9	27.6 ± 12.1	3.52 ± 0.52
2003	26	71.0 ± 10.6	79.7 ± 17.9	3.39 ± 0.43	80.1 ± 10.0	10.5 ± 5.2	3.65 ± 0.62
2009	5	76.0 ± 7.1	88.0 ± 20.3	3.36 0.29	83.4 ± 6.8	16.0 ± 7.5	3.03 ± 0.36

FIGURES

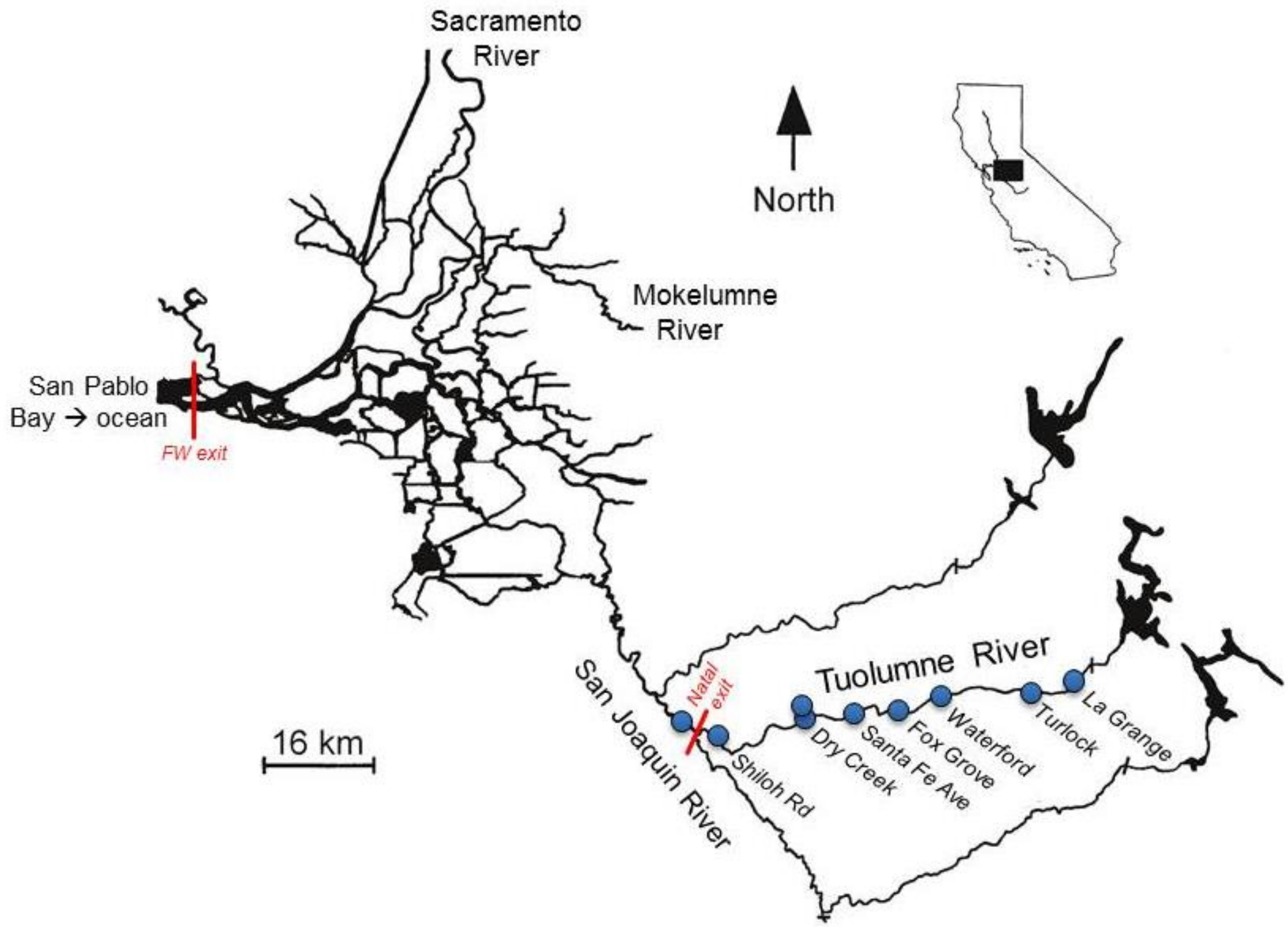


Fig. 1 Map to show location of the Tuolumne and San Joaquin rivers, and the sites sampled for water isotope analyses as part of a different project (blue circles; A. Sturrock, unpublished). The locations defined as natal and freshwater (FW) exit are indicated by red lines.

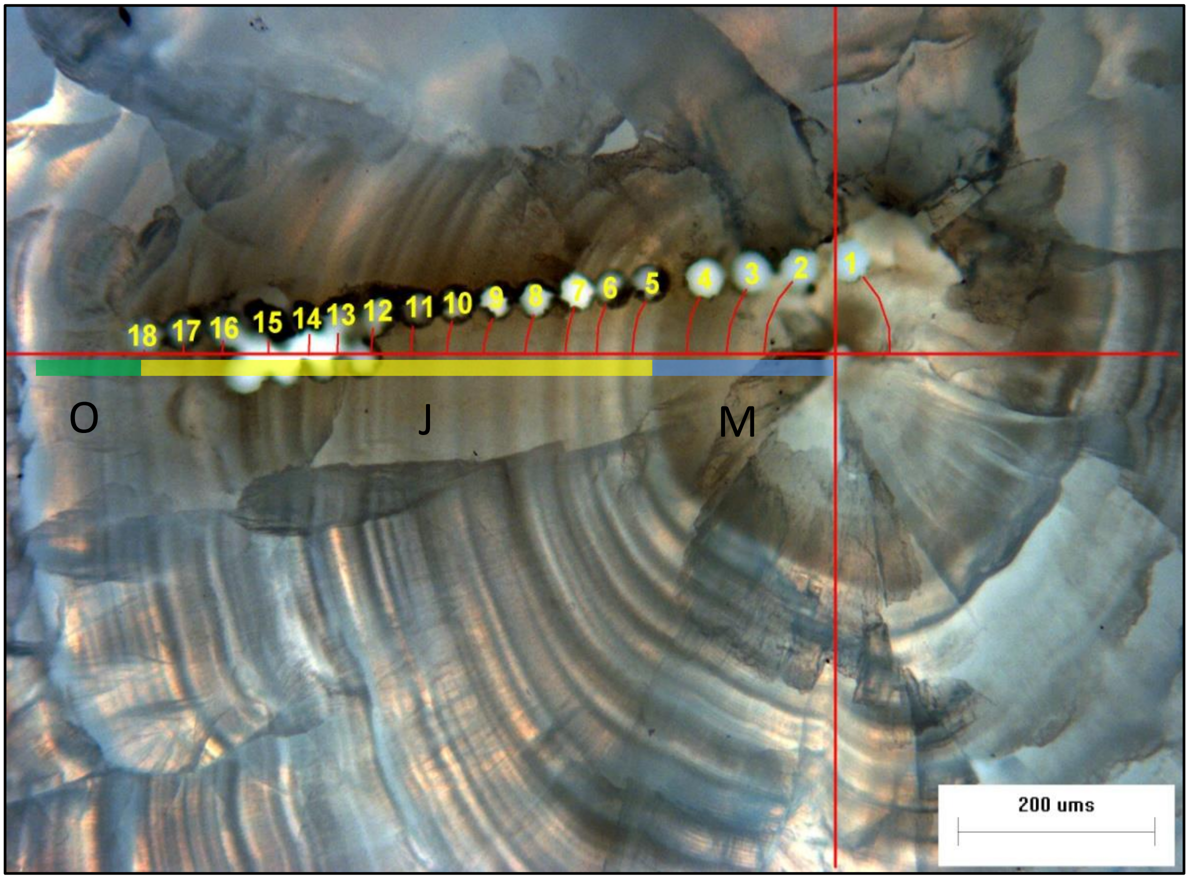


Fig. 2 A typical $^{87}Sr/^{86}Sr$ transect showing spot analyses (numbered) from the core to ocean entry. The life history stages are indicated by letters: maternal (M), juvenile (J) and ocean (O). The distance at which the final 'natal spot' intersected the 90° transect (indicated by curved red lines) was used to back-calculate size at outmigration. Note the 'respots' at positions 12.5 to 15.5 (located under the yellow bar) used to more accurately identify exit point.

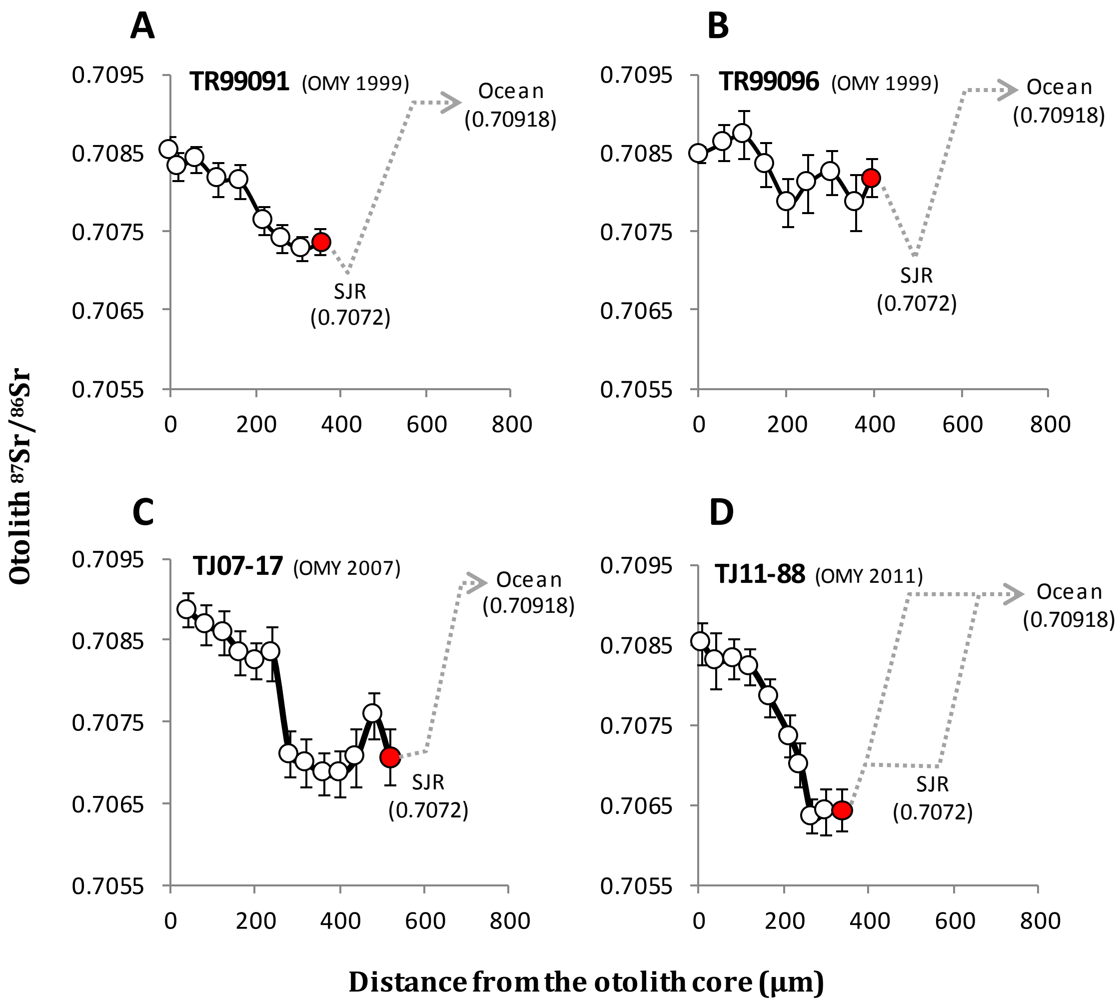


Fig. 3 Otolith $^{87}Sr/^{86}Sr$ profiles from four juvenile salmon captured in the lower Tuolumne River in outmigration years (OMY) 1999, 2007 and 2011. The natal exit spot ("last natal value") is indicated in red, along with the expected profile trajectory (dotted lines) through the San Joaquin River (SJR) to the ocean, had the fish not been captured as a juvenile and was instead being sampled as a returning adult. Note that the juvenile in plot D had moved to the lower river (or Dry Creek) immediately after emergence (~250um from the core) and the dotted lines indicate two possible trajectories, one with extended rearing in the SJR prior to leaving freshwater and the other with direct outmigration to the ocean.

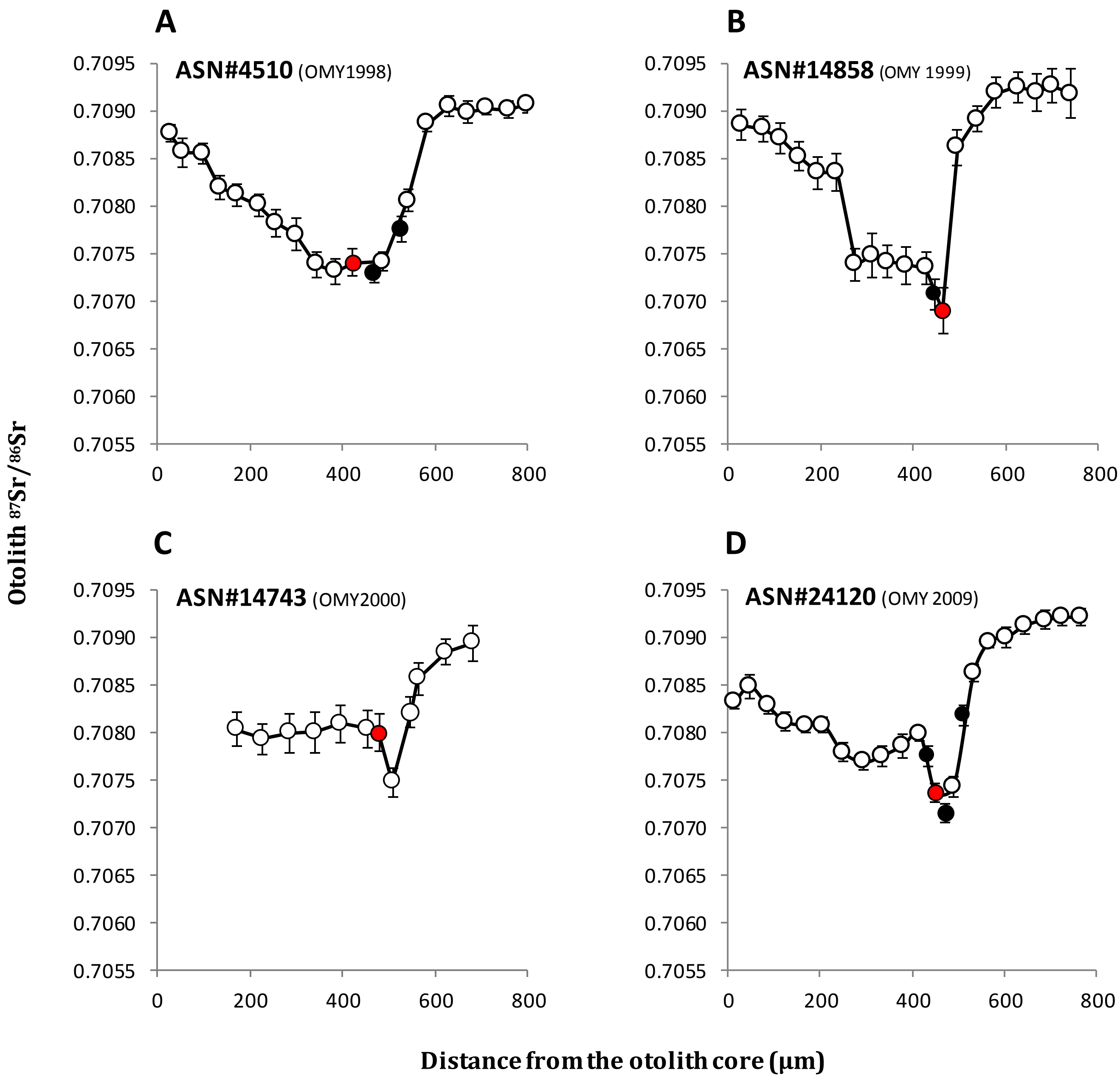


Fig. 4 Examples of otolith $^{87}Sr/^{86}Sr$ profiles from adult salmon carcasses collected in the lower Tuolumne River that were assigned to the Tuolumne River, having outmigrated as juveniles in 1998-2009. The inferred 'last natal spot' prior to outmigration to the SJR and ocean is shown in red. Black symbols indicate respots.

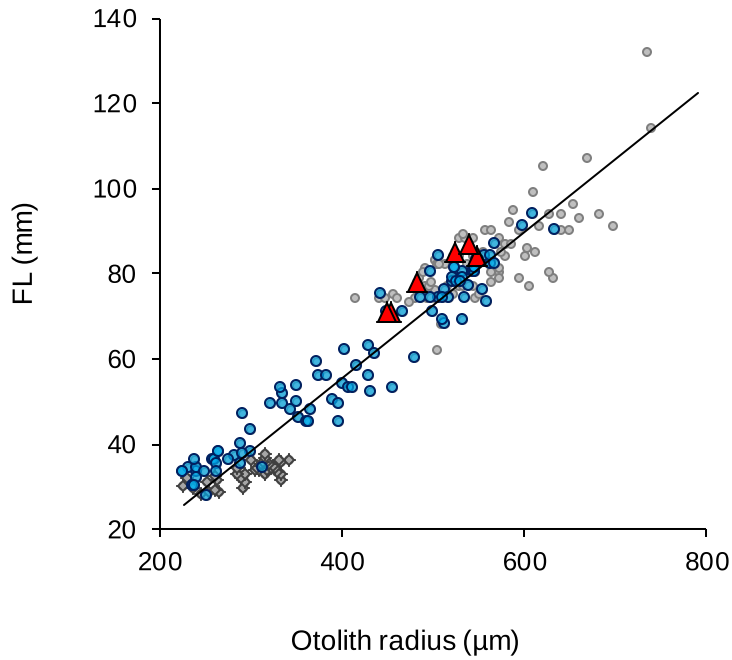


Fig. 5 Relationship between otolith radius and fork length (FL) of juveniles of known origin (Sturrock, unpublished) (n=224, $r^2 = 0.92$) used to reconstruct size at outmigration in returning adults from the current study. The 224 reference samples are all in the same Evolutionary Significant Unit (California Central Valley fall run salmon) and include individuals from the Tuolumne River (n=6; red triangles), the Stanislaus River (n=95; blue circles), Coleman National Fish Hatchery (n=40; grey diamonds) and the San Francisco Bay at Golden Gate Bridge of unknown origin within the CCV (n=83; grey circles).

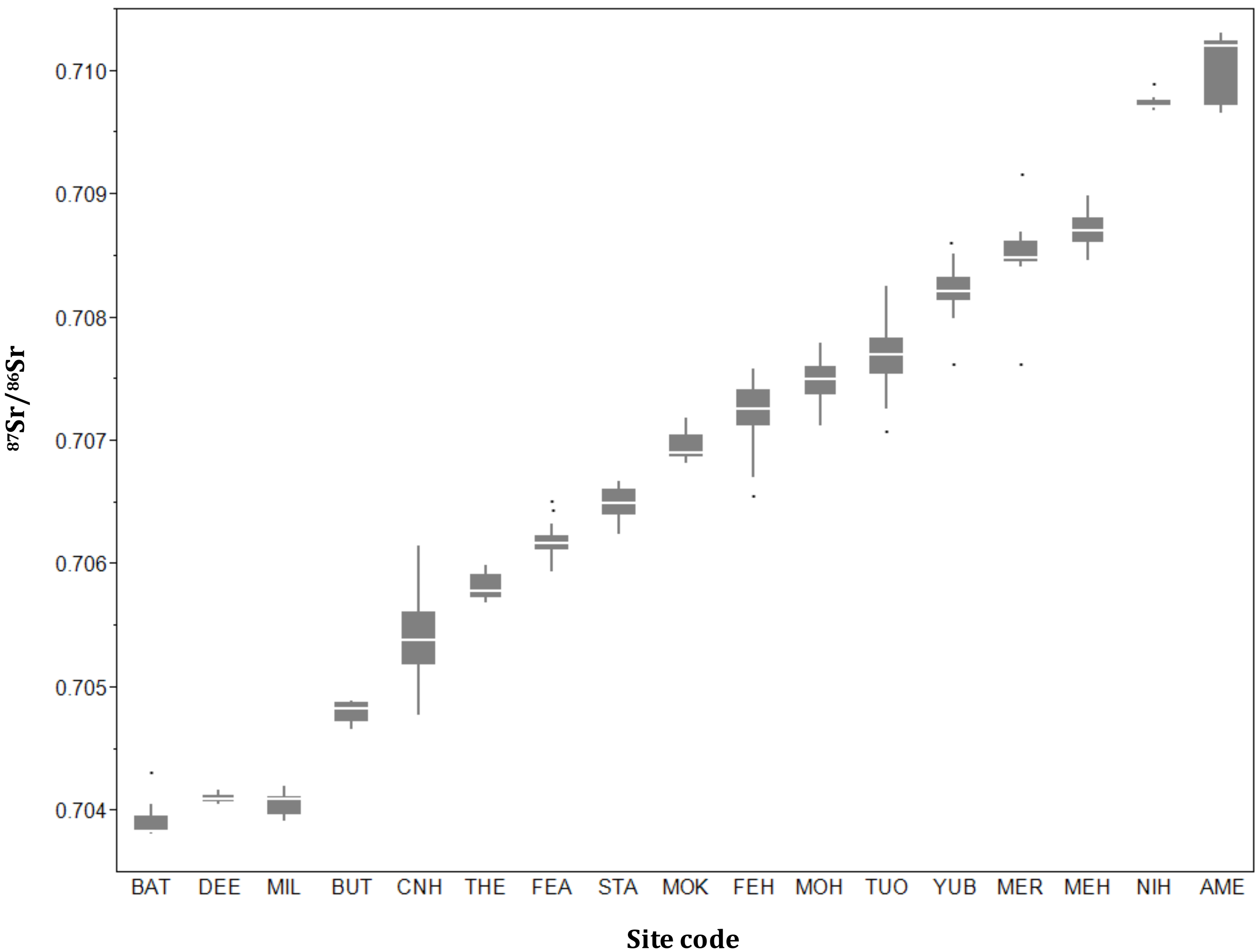


Fig. 6 Differences in $^{87}Sr/^{86}Sr$ values among sites in the CCV, modified from [1] using additional water samples and otoliths from known-origin juveniles and adult CWT fish analyzed as part of existing and ongoing projects ([34], P. Weber & A. Sturrock, unpublished). Site codes identified in Table 2. These data were used to predict the origin of adults collected in the current study. Due to overlap among TUO, MOH and FEH, all fish identified as potentially originated in the Tuolumne River (TUO) using Sr isotopes were also assigned to hatchery/wild using otolith microstructure (Barnett-Johnson et al., 2007).

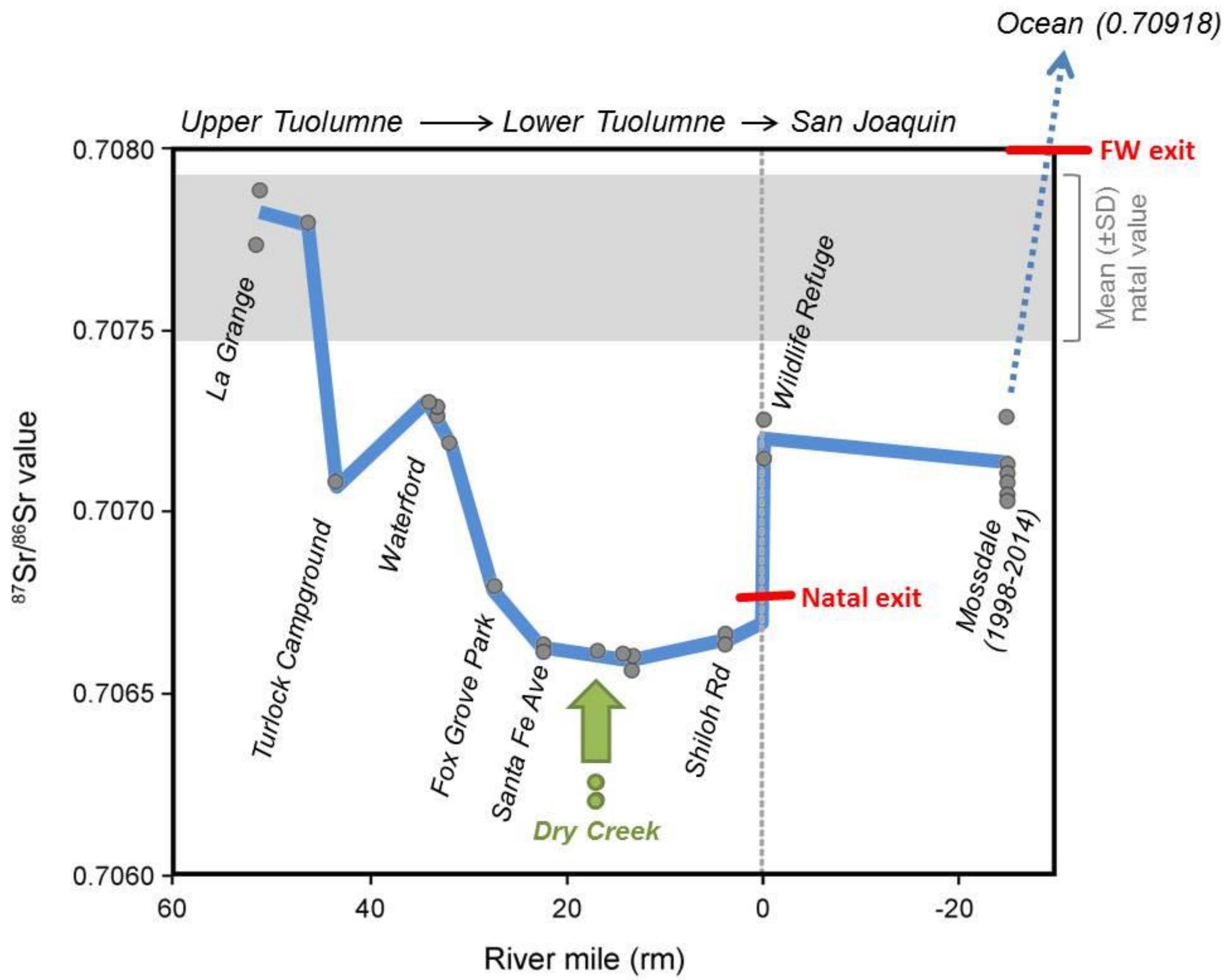


Fig. 7 Trends in water $^{87}Sr/^{86}Sr$ in the mainstem Tuolumne and San Joaquin Rivers (samples collected as part of other studies). The majority of measurements were collected in January and February 2014; however, additional years are included where available. The shaded grey bar indicates the mean natal value allocated to the Tuolumne (±SD), based on otolith analyses of juveniles captured in a rotary screw trap close to Shiloh Road (i.e., prior to outmigration). The blue trend line within the Tuolumne River is driven by sources of isotopically light water entering the river downstream of the spawning reaches (~rm50). At the time of writing, Dry Creek (rm 16.7) is the only known example of such a source.

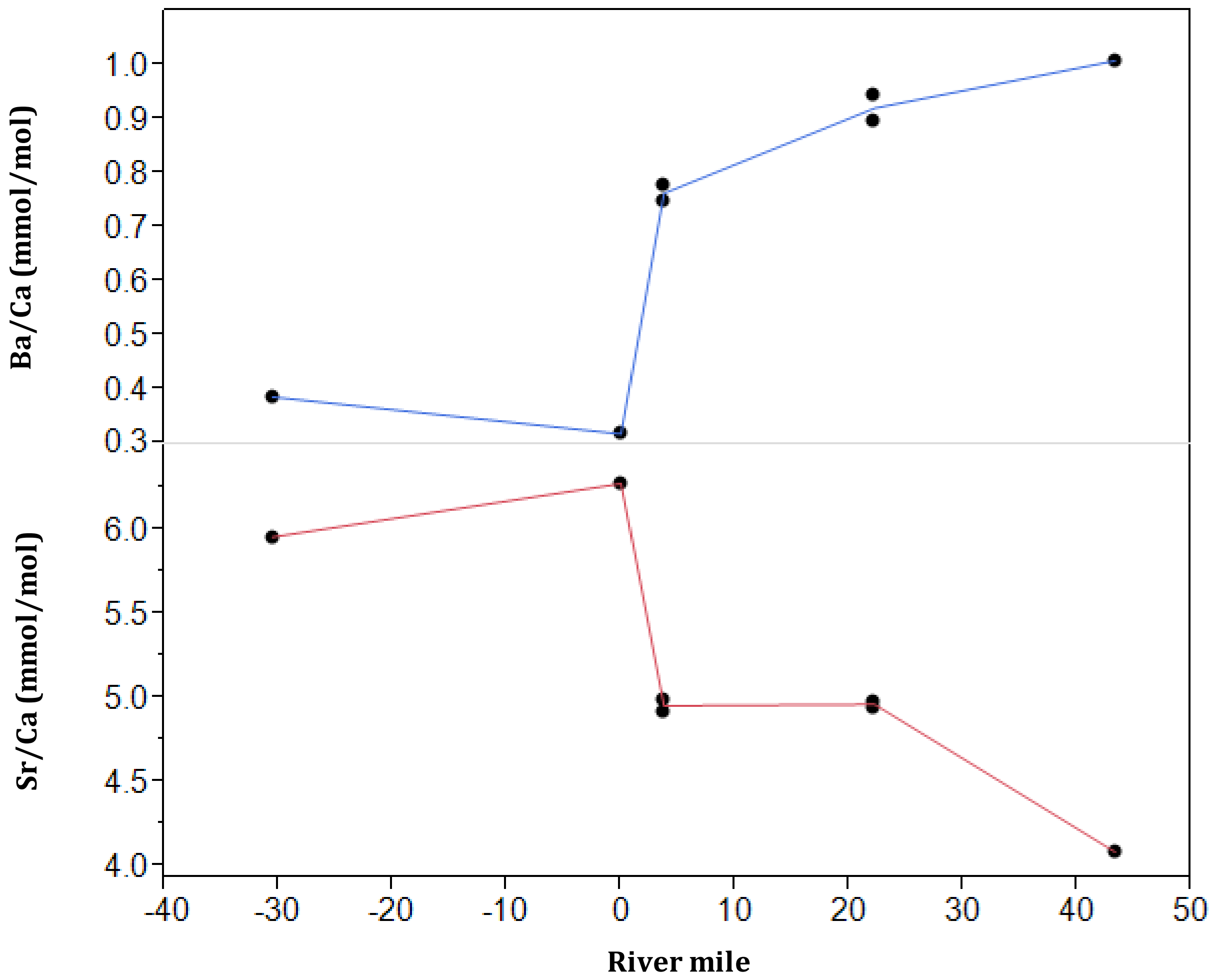
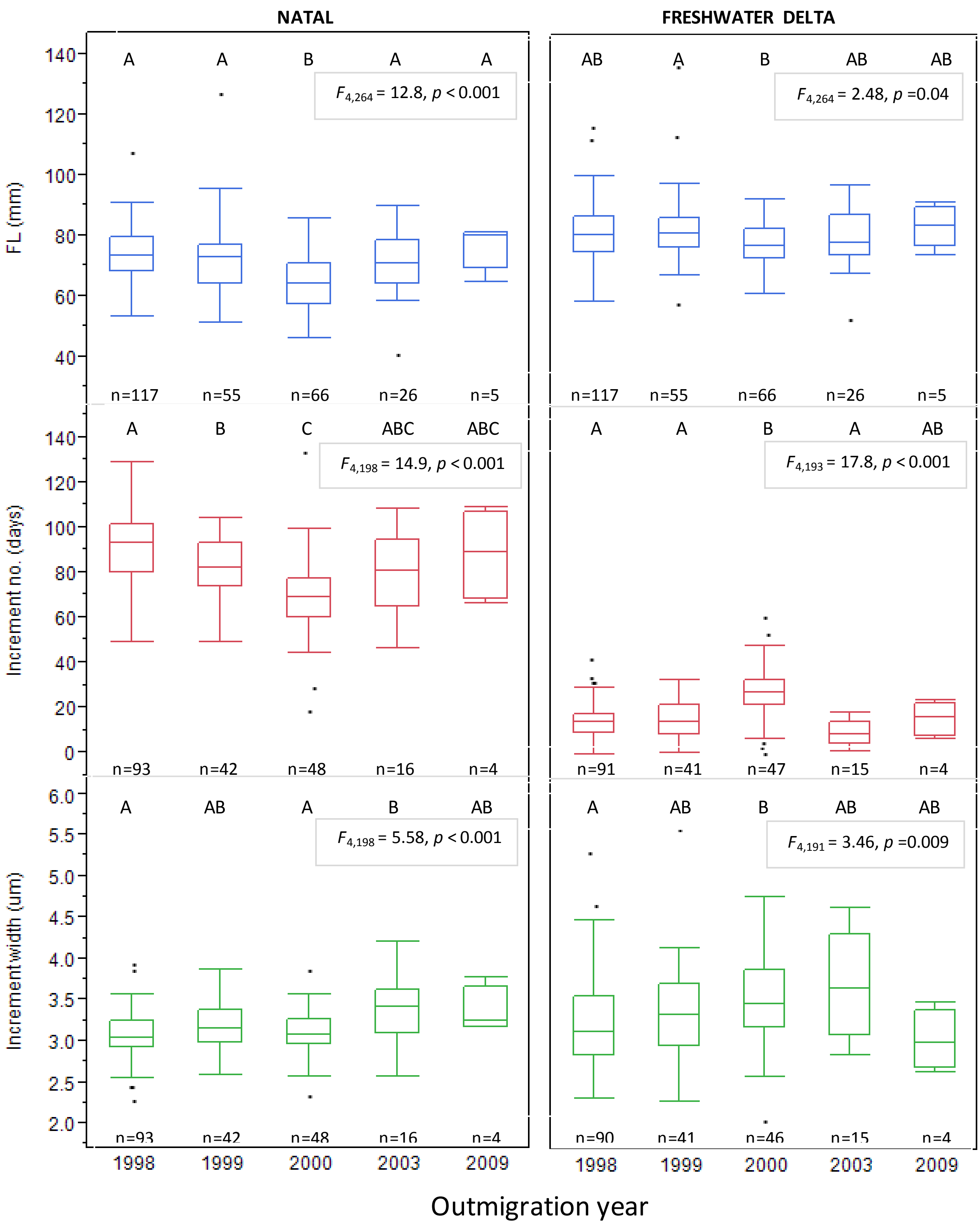


Fig. 8 Trends in water Ba/Ca and Sr/Ca between the Tuolumne and San Joaquin rivers (samples collected as part of other studies). Note the sharp inflection between the lower Tuolumne (~river mile 3) and the San Joaquin (river mile 0) rivers.

Fig. 9 Trends in median fork length at exit (FL), number of otolith increments (age) and increment width (growth rate) in the natal river (left) and freshwater delta (right) of juveniles that originated in and returned to the Tuolumne River. Overall differences among years were tested by ANOVA (results exhibited on each plot). Bars not connected by the same letter are significantly different (p<0.05, Tukey's test).



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Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

						Natal Sr ratio				Natal exit	Predicted FL at natal exit (mm)			Predicted life stage at natal exit [3]				Increment width (um)		
Sample ID	Capture date	Capture FL (cm)	Scale age	Sex	Outmi-gration year	Mean natal value	Prob to TUO [1]	H vs. W [2]	Natal location	Otolith distance (um)	FL	Lower 95% CI	Upper 95% CI	Life stage	Lower 95% CI	Upper 95% CI	Increment no (days)	Mean	CV	Notes
4175	10/10/00	98	3	M	1998	0.70799	0.97	W	TUO	576.6	85.8	77.2	95.9	S	S	S	109	3.26	0.19	
4176	10/10/00	91	3	M	1998	0.70774	0.95	W	TUO	544.4	80.3	71.7	90.4	S	P	S	96	3.53	0.21	
4182	10/17/00	76	3	M	1998	0.70803	0.96	W	TUO	544.1	80.3	71.7	90.3	S	P	S	112	2.96	0.19	
4183	10/17/00	90	3	M	1998	0.70797	0.97	W	TUO	494.3	71.8	63.2	81.8	P	P	S	94	2.74	0.22	
4185	10/17/00	84	3	F	1998	0.70728	0.64	W	TUO	522.7	76.6	68.0	86.7	S	P	S	107	3.17	0.26	
4189	10/24/00	90	3	F	1998	0.70806	0.94	W	TUO	452.6	64.6	56.1	74.7	P	P	P	83	2.90	0.23	
4192	10/24/00	87.5	3	F	1998	0.70807	0.93	W	TUO	487.5	70.6	62.0	80.7	P	P	S	87	3.22	0.23	
4196	10/24/00	67.9	3	F	1998	0.70800	0.97	W	TUO	493.3	71.6	63.0	81.7	P	P	S	98 †	2.62	0.20	† Microstructure ran out 33um before last natal spot (inferred 12 increments)
4197	10/24/00	78.6	3	F	1998	0.70740	0.61	W	TUO	423.2	59.6	51.0	69.7	P	F	P	79	2.74	0.26	
4200	10/24/00	68.6	3	F	1998	0.70760	0.88	W	TUO	569.4	84.6	76.0	94.7	S	S	S	102	3.20	0.27	
4210	10/24/00	88.3	3	M	1998	0.70764	0.91	W	TUO	488.9	70.8	62.2	80.9	P	P	S	93	2.98	0.28	
4211	10/24/00	72	3	F	1998	0.70783	0.97	W	TUO	581.0	86.6	78.0	96.6	S	S	S	.			
4212	10/24/00	78.1	3	M	1998	0.70765	0.91	W	TUO	541.0	79.7	71.2	89.8	S	P	S	93	2.93	0.20	
4215	10/25/00	79	3	F	1998	0.70802	0.96	W	TUO	552.5	81.7	73.1	91.8	S	P	S	111	3.03	0.20	
4226	10/25/00	80	3	F	1998	0.70770	0.93	W	TUO	446.6	63.6	55.0	73.7	P	P	P	70	3.11	0.33	
4232	10/25/00	88.5	3	M	1998	0.70821	0.53	W	TUO	519.3	76.0	67.5	86.1	S	P	S	.			
4233	10/25/00	72	3	F	1998	0.70795	0.98	W	TUO	452.6	64.6	56.1	74.7	P	P	P	74	3.02	0.20	
4234	10/25/00	77	3	F	1998	0.70823	0.45	W	TUO*	579.9	86.4	77.8	96.5	S	S	S	*Inconclusive natal assignment*			
4240	10/26/00	80	3	F	1998	0.70726	0.28	W	TUO*	426.2	60.1	51.5	70.2	P	F	P	*Inconclusive natal assignment*			
4249	10/30/00	80	3	F	1998	0.70737	0.54	W	TUO	485.8	70.3	61.7	80.4	P	P	S	.			
4253	10/30/00	80	3	M	1998	0.70810	0.90	W	TUO	554.2	82.0	73.4	92.1	S	P	S	101	3.05	0.26	
4266	10/30/00	77	3	F	1998	0.70740	0.60	W	TUO	461.1	66.1	57.5	76.2	P	P	S	100	2.43	0.25	
4267	10/30/00	75	3	F	1998	0.70812	0.86	W	TUO	476.3	68.7	60.1	78.8	P	P	S	.			
4269	10/30/00	80	3	F	1998	0.70732	0.43	W	TUO*	581.6	86.7	78.1	96.8	S	S	S	*Inconclusive natal assignment*			
4275	10/31/00	79	3	F	1998	0.70721	0.18	W	TUO*	416.1	58.4	49.8	68.5	P	F	P	*Inconclusive natal assignment*			
4278	10/31/00	83	3	F	1998	0.70802	0.96	W	TUO	480.1	69.3	60.7	79.4	P	P	S	.			
4279	10/31/00	87.5	3	F	1998	0.70798	0.97	W	TUO	568.8	84.5	75.9	94.6	S	S	S	120	3.18	0.22	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

4281	10/31/00	91	3	M	1998	0.70728	0.31	W	TUO*	454.0	64.9	56.3	74.9	P	P	P	*Inconclusive natal assignment*			
4292	10/31/00	74	3	F	1998	0.70733	0.44	W	TUO*	526.1	77.2	68.6	87.3	S	P	S	*Inconclusive natal assignment*			
4294	10/31/00	86	3	F	1998	0.70800	0.97	W	TUO	560.6	83.1	74.5	93.2	S	P	S	.			
4295	10/31/00	72	3	F	1998	0.70816	0.77	W	TUO	495.0	71.9	63.3	81.9	P	P	S	87	3.13	0.23	
4297	11/01/00	74	3	F	1998	0.70805	0.95	W	TUO	576.9	85.9	77.3	96.0	S	S	S	109	2.98	0.18	
4299	11/06/00	81	3	F	1998	0.70801	0.97	W	TUO	527.8	77.5	68.9	87.6	S	P	S	.			
4300	11/06/00	96	3	M	1998	0.70735	0.50	W	TUO*	452.0	64.5	55.9	74.6	P	P	P	*Inconclusive natal assignment*			
4306	11/06/00	85	3	F	1998	0.70801	0.97	W	TUO	520.7	76.3	67.7	86.3	S	P	S	93	3.27	0.17	
4309	11/06/00	84	3	F	1998	0.70807	0.93	W	TUO	453.7	64.8	56.2	74.9	P	P	P	.			
4311	11/06/00	74	3	F	1998	0.70752	0.81	W	TUO	432.7	61.2	52.6	71.3	P	F	P	73	3.03	0.22	
4316	11/06/00	81	3	F	1998	0.70738	0.55	W	TUO	383.9	52.9	44.3	63.0	F	F	P	.			
4317	11/06/00	79	3	F	1998	0.70786	0.97	W	TUO	488.2	70.7	62.1	80.8	P	P	S	94 †	2.82	0.26	† Microstructure ran out 55um before last natal spot (inferred 19 increments)
4321	11/06/00	70	3	F	1998	0.70742	0.65	W	TUO	500.4	72.8	64.2	82.9	P	P	S	73	3.54	0.36	
4331	11/07/00	86	3	M	1998	0.70798	0.97	W	TUO	571.5	85.0	76.4	95.0	S	S	S	127	3.00	0.20	
4334	11/07/00	85	3	F	1998	0.70739	0.59	W	TUO	384.6	53.0	44.4	63.1	F	F	P	54	3.44	0.23	
4337	11/07/00	74	3	F	1998	0.70733	0.45	W	TUO*	535.3	78.8	70.2	88.8	S	P	S	*Inconclusive natal assignment*			
4340	11/07/00	75.5	3	F	1998	0.70783	0.97	W	TUO	490.2	71.1	62.5	81.1	P	P	S	.			
4343	11/07/00	81	3	F	1998	0.70768	0.92	W	TUO	509.5	74.4	65.8	84.4	P	P	S	109	2.96	0.26	
4352	11/07/00	73	3	F	1998	0.70788	0.97	W	TUO	563.0	83.5	74.9	93.6	S	P	S	81	3.09	0.20	
4360	11/08/00	76.5	3	F	1998	0.70818	0.67	W	TUO	578.2	86.1	77.5	96.2	S	S	S	.			
4376	11/09/00	85	3	F	1998	0.70733	0.46	W	TUO*	571.8	85.0	76.4	95.1	S	S	S	*Inconclusive natal assignment*			
4378	11/09/00	88	3	M	1998	0.70728	0.33	W	TUO*	607.4	91.1	82.5	101.2	S	S	S	*Inconclusive natal assignment*			
4381	11/13/00	90	3	M	1998	0.70816	0.75	W	TUO	455.0	65.0	56.5	75.1	P	P	S	75	3.02	0.20	
4383	11/13/00	79	3	M	1998	0.70756	0.85	W	TUO	529.2	77.7	69.1	87.8	S	P	S	.			
4384	11/13/00	80	3	F	1998	0.70786	0.97	W	TUO	506.8	73.9	65.3	84.0	P	P	S	85	3.16	0.23	
4397	11/13/00	67	3	F	1998	0.70819	0.66	W	TUO	474.7	68.4	59.8	78.5	P	P	S	84	2.93	0.26	
4403	11/14/00	77	3	F	1998	0.70808	0.92	W	TUO	501.7	73.0	64.4	83.1	P	P	S	90	2.99	0.23	
4414	11/14/00	81	3	F	1998	0.70749	0.77	W	TUO	467.5	67.2	58.6	77.3	P	P	S	.			
4418	11/14/00	86	3	F	1998	0.70742	0.66	W	TUO	460.1	65.9	57.3	76.0	P	P	S	65	3.84	0.34	
4424	11/14/00	77	3	F	1998	0.70783	0.97	W	TUO	552.9	81.8	73.2	91.8	S	P	S	125	2.95	0.21	
4441	11/20/00	72	3	F	1998	0.70823	0.45	W	TUO*	485.5	70.3	61.7	80.3	P	P	S	*Inconclusive natal assignment*			

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

4442	11/20/00	95	3	M	1998	0.70771	0.94	W	TUO	592.1	88.5	79.9	98.6	S	S	S	114	3.25	0.28	
4443	11/20/00	100	3	M	1998	0.70735	0.50	W	TUO*	475.3	68.5	59.9	78.6	P	P	S	*Inconclusive natal assignment*			
4450	11/20/00	82	3	F	1998	0.70817	0.73	W	TUO	447.6	63.8	55.2	73.8	P	P	P	104	2.26	0.24	
4451	11/20/00	92	3	M	1998	0.70817	0.72	W	TUO	472.3	68.0	59.4	78.1	P	P	S	108	2.82	0.25	
4455	11/20/00	74	3	F	1998	0.70769	0.93	W	TUO	514.6	75.2	66.6	85.3	S	P	S	78	3.29	0.22	
4458	11/21/00	80	3	F	1998	0.70792	0.98	W	TUO	543.4	80.2	71.6	90.2	S	P	S	92	3.33	0.26	
4476	11/22/00	100	3	M	1998	0.70804	0.95	W	TUO	514.9	75.3	66.7	85.4	S	P	S	111	2.68	0.32	
4484	11/27/00	77	3	F	1998	0.70788	0.97	W	TUO	528.8	77.7	69.1	87.7	S	P	S	90	3.06	0.26	
4487	11/27/00	84	3	F	1998	0.70800	0.97	W	TUO	493.6	71.6	63.1	81.7	P	P	S	.			
4504	12/04/00	100	3	M	1998	0.70826	0.30	W	TUO*	480.4	69.4	60.8	79.5	P	P	S	*Inconclusive natal assignment*			
4506	12/04/00	80	3	F	1998	0.70756	0.85	W	TUO	406.6	56.8	48.2	66.8	P	F	P	60	3.23	0.24	
4508	12/04/00	89	3	F	1998	0.70806	0.94	W	TUO	466.5	67.0	58.4	77.1	P	P	S	85	2.76	0.18	
4509	12/04/00	70.5	3	F	1998	0.70812	0.86	W	TUO	489.9	71.0	62.4	81.1	P	P	S	91	3.14	0.22	
4510	12/05/00	77	3	F	1998	0.70776	0.95	W	TUO	422.9	59.5	51.0	69.6	P	F	P	49	3.30	0.28	
4514	12/05/00	78	3	F	1998	0.70794	0.98	W	TUO	462.1	66.3	57.7	76.3	P	P	S	.			
4515	12/05/00	77	3	F	1998	0.70815	0.80	W	TUO	481.1	69.5	60.9	79.6	P	P	S	80	3.39	0.21	
4516	12/05/00	82	3	F	1998	0.70818	0.69	W	TUO	471.3	67.8	59.2	77.9	P	P	S	75	3.31	0.22	
4517	12/05/00	88.5	3	F	1998	0.70798	0.97	W	TUO	526.8	77.3	68.7	87.4	S	P	S	95	3.04	0.20	
4518	12/05/00	83	3	F	1998	0.70789	0.97	W	TUO	519.3	76.0	67.5	86.1	S	P	S	94	3.30	0.24	
4521	12/06/00	78.5	3	F	1998	0.70788	0.97	W	TUO	537.6	79.2	70.6	89.2	S	P	S	83	3.24	0.22	
4527	12/11/00	83	3	M	1998	0.70819	0.66	W	TUO	543.4	80.2	71.6	90.2	S	P	S	93	3.40	0.27	
4535	12/19/00	78	3	F	1998	0.70814	0.82	W	TUO	503.1	73.3	64.7	83.3	P	P	S	100	3.14	0.22	
9536	07/07/00	75	3	F	1998	0.70775	0.95	W	TUO	700.5	107.0	98.4	117.1	S	S	S	.			
11015	11/16/01	86.5	4	F	1998	0.70789	0.97	W	TUO	486.3	70.4	61.8	80.5	P	P	S	80	3.53	0.28	
11036	12/11/01	86	4	F	1998	0.70772	0.94	W	TUO	580.3	86.5	77.9	96.5	S	S	S	95	3.56	0.21	
11037	12/11/01	110	4	M	1998	0.70792	0.98	W	TUO	538.6	79.3	70.8	89.4	S	P	S	93	3.02	0.27	
11038	12/11/01	78	4	F	1998	0.70821	0.53	W	TUO	477.7	68.9	60.3	79.0	P	P	S	.			
11040	12/11/01	98	4	F	1998	0.70812	0.86	W	TUO	549.8	81.3	72.7	91.3	S	P	S	114	3.02	0.30	
11056	11/20/01	78	4	F	1998	0.70779	0.96	W	TUO	504.4	73.5	64.9	83.6	P	P	S	115	2.67	0.23	
11064	11/20/01	95	4	M	1998	0.70745	0.71	W	TUO	541.0	79.7	71.2	89.8	S	P	S	92	3.92	0.34	
11072	11/20/01	112	4	M	1998	0.70816	0.75	W	TUO	468.6	67.4	58.8	77.4	P	P	S	75	3.05	0.19	
11085	11/20/01	87	4	F	1998	0.70737	0.55	W	TUO	411.3	57.6	49.0	67.6	P	F	P	.			
11089	11/20/01	104	4	M	1998	0.70816	0.76	W	TUO	479.1	69.2	60.6	79.2	P	P	S	90	2.79	0.19	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

11097	11/30/01	82	4	M	1998	0.70743	0.67	W	TUO	518.3	75.9	67.3	85.9	S	P	S	88	3.23	0.39	
11098	11/30/01	87	4	F	1998	0.70807	0.93	W	TUO	530.2	77.9	69.3	88.0	S	P	S	93	3.20	0.20	
11140	11/26/01	88	4	F	1998	0.70769	0.93	W	TUO	479.4	69.2	60.6	79.3	P	P	S	79	2.90	0.27	
11154	11/26/01	87	4	F	1998	0.70794	0.98	W	TUO	518.7	75.9	67.3	86.0	S	P	S	91	3.25	0.27	
11176	12/07/01	92.5	4	F	1998	0.70821	0.54	W	TUO	544.7	80.4	71.8	90.5	S	P	S	.			
11177	12/07/01	90	4	F	1998	0.70781	0.96	W	TUO	484.1	70.0	61.4	80.1	P	P	S	.			
11181	12/18/01	87	3	F	1998	0.70819	0.63	W	TUO	604.7	90.6	82.0	100.7	S	S	S	129	3.21	0.22	
11182	12/17/01	99	4	M	1998	0.70798	0.97	W	TUO	397.8	55.3	46.7	65.3	P	F	P	51	3.10	0.23	
11190	11/23/01	90	3	M	1998	0.70821	0.53	W	TUO	506.8	73.9	65.3	84.0	P	P	S	95	2.99	0.24	
11216	11/21/01	94	4	F	1998	0.70821	0.54	W	TUO	517.6	75.8	67.2	85.8	S	P	S	122	2.55	0.27	
19680	11/15/01	103	4	M	1998	0.70766	0.92	W	TUO	603.0	90.3	81.8	100.4	S	S	S	.			
19684	11/15/01	92	3.5	M	1998	0.70776	0.95	W	TUO	508.2	74.1	65.5	84.2	P	P	S	.			
19685	11/15/01	87	4	F	1998	0.70811	0.89	W	TUO	513.2	75.0	66.4	85.1	S	P	S	.			
19687	11/15/01	82	3.5	M	1998	0.70806	0.95	W	TUO	515.3	75.4	66.8	85.4	S	P	S	96	2.97	0.21	
19691	11/15/01	91	4	M	1998	0.70721	0.19	W	TUO*	421.8	59.4	50.8	69.4	P	F	P	*Inconclusive natal assignment*			
19719	11/19/01	94.5	3.5	F	1998	0.70806	0.94	W	TUO	510.5	74.5	66.0	84.6	P	P	S	89	2.88	0.23	
19772	11/28/01	97	4	F	1998	0.70769	0.93	W	TUO	467.9	67.2	58.7	77.3	P	P	S	84	2.81	0.24	
19776	11/28/01	90	4	F	1998	0.70821	0.53	W	TUO	522.7	76.6	68.0	86.7	S	P	S	101	3.10	0.19	
19777	11/28/01	91	4	F	1998	0.70816	0.76	W	TUO	489.2	70.9	62.3	81.0	P	P	S	96	2.67	0.30	
19781	11/28/01	86	4	F	1998	0.70824	0.37	W	TUO*	532.5	78.3	69.7	88.4	S	P	S	*Inconclusive natal assignment*			
19783	11/28/01	89	4	F	1998	0.70793	0.98	W	TUO	432.7	61.2	52.6	71.3	P	F	P	68	3.03	0.26	
19785	11/28/01	88	4	F	1998	0.70765	0.91	W	TUO	446.2	63.5	55.0	73.6	P	F	P	67	3.16	0.25	
19790	11/28/01	94	4	F	1998	0.70818	0.68	W	TUO	504.1	73.4	64.9	83.5	P	P	S	104	2.44	0.24	
19796	12/03/01	81	4	F	1998	0.70811	0.88	W	TUO	509.9	74.4	65.8	84.5	P	P	S	83	3.31	0.24	
19798	12/03/01	93	4	M	1998	0.70814	0.83	W	TUO	474.7	68.4	59.8	78.5	P	P	S	101	3.02	0.23	
19800	12/03/01	114	4	M	1998	0.70776	0.95	W	TUO	569.8	84.7	76.1	94.7	S	S	S	.			
19802	12/03/01	97	4	F	1998	0.70824	0.40	W	TUO*	539.3	79.5	70.9	89.5	S	P	S	*Inconclusive natal assignment*			
19805	12/03/01	88	4	F	1998	0.70821	0.56	W	TUO	496.7	72.2	63.6	82.2	P	P	S	95	3.15	0.25	
19806	12/03/01	89	4	F	1998	0.70769	0.93	W	TUO	542.4	80.0	71.4	90.0	S	P	S	103	3.04	0.28	
19810	12/03/01	85	4	F	1998	0.70765	0.91	W	TUO	479.1	69.2	60.6	79.2	P	P	S	74	3.56	0.28	
19820	12/03/01	105	4	M	1998	0.70779	0.96	W	TUO	492.6	71.5	62.9	81.5	P	P	S	88	3.37	0.28	
19821	12/03/01	94	4	F	1998	0.70812	0.86	W	TUO	494.3	71.8	63.2	81.8	P	P	S	95	2.63	0.30	
19838	12/03/01	73	4	F	1998	0.70765	0.91	W	TUO	462.1	66.3	57.7	76.3	P	P	S	77	3.16	0.24	

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19840	12/03/01	81	4	F	1998	0.70813	0.84	W	TUO	436.1	61.8	53.2	71.9	P	F	P	84	2.87	0.24	
19857	12/04/01	97	4	M	1998	0.70776	0.95	W	TUO	538.0	79.2	70.6	89.3	S	P	S	93	3.20	0.20	
19864	12/04/01	99	4	M	1998	0.70813	0.84	W	TUO	514.9	75.3	66.7	85.4	S	P	S	109	3.02	0.27	
19867	12/04/01	86	4	F	1998	0.70808	0.93	W	TUO	478.7	69.1	60.5	79.2	P	P	S	95	2.99	0.26	
19872	12/10/01	84	4	F	1998	0.70815	0.78	W	TUO	463.1	66.4	57.8	76.5	P	P	S	77	2.88	0.19	
19875	12/10/01	83	4	F	1998	0.70823	0.42	W	TUO*	478.7	69.1	60.5	79.2	P	P	S	*Inconclusive natal assignment*			
19879	12/10/01	76	4	F	1998	0.70766	0.91	W	TUO	464.2	66.6	58.0	76.7	P	P	S	84	3.01	0.21	
19880	12/10/01	89	4	F	1998	0.70765	0.91	W	TUO	447.6	63.8	55.2	73.8	P	P	P	72	2.95	0.23	
19881	12/10/01	99	4	F	1998	0.70813	0.85	W	TUO	528.5	77.6	69.0	87.7	S	P	S	73	2.85	0.21	
20183	11/28/01	101	4	F	1998	0.70773	0.94	W	TUO	525.4	77.1	68.5	87.2	S	P	S	98	3.30	0.31	
4492	11/28/00	57	2	M	1999	0.70804	0.95	W	TUO	578.6	86.2	77.6	96.2	S	S	S	104 †	3.21	0.25	† Microstructure ran out 18um before last natal spot (inferred 5 increments)
4526	12/11/00	67	2	F	1999	0.70800	0.97	W	TUO	815.6	126.7	118.1	136.8	S	S	S	.			
11009	11/16/01	.	3	F	1999	0.70757	0.86	W	TUO	441.7	62.8	54.2	72.8	P	F	P	.			
11016	11/16/01	79.5	3	F	1999	0.70731	0.40	W	TUO*	369.6	50.4	41.9	60.5	F	F	P	49	2.84	0.25	
11019	11/16/01	60	3	F	1999	0.70743	0.67	INC	TUO*	476.5	68.7	60.1	78.8	P	P	S	.			Unreadable, so cannot assign natal location or do ageing
11021	11/16/01	73	3	F	1999	0.70731	0.39	W	TUO*	478.8	69.1	60.5	79.2	P	P	S	84	2.75	0.21	
11041	12/11/01	77	3	F	1999	0.70805	0.95	W	TUO	509.5	74.4	65.8	84.4	P	P	S	93	2.97	0.24	
11094	11/30/01	80	3	F	1999	0.70756	0.85	W	TUO	444.0	63.2	54.6	73.2	P	F	P	64	2.98	0.29	
11096	11/30/01	77	3	F	1999	0.70764	0.91	W	TUO	503.0	73.3	64.7	83.3	P	P	S	97	2.94	0.23	
11099	11/30/01	73	3	F	1999	0.70733	0.44	W	TUO*	467.2	67.1	58.5	77.2	P	P	S	*Inconclusive natal assignment*			
11100	11/30/01	83	3	F	1999	0.70748	0.75	W	TUO	442.6	62.9	54.3	73.0	P	F	P	.			
11132	11/26/01	76	3	F	1999	0.70734	0.46	W	TUO*	462.1	66.3	57.7	76.3	P	P	S	91	2.91	0.24	
11141	11/26/01	77	3	F	1999	0.70741	0.63	W	TUO	395.6	54.9	46.3	65.0	F	F	P	.			
11146	11/26/01	81	3	F	1999	0.70722	0.20	W	TUO*	434.2	61.5	52.9	71.6	P	F	P	*Inconclusive natal assignment*			
11157	11/26/01	80	3	M	1999	0.70740	0.61	W	TUO	405.4	56.6	48.0	66.6	P	F	P	64	3.00	0.32	
11161	11/26/01	74	3	F	1999	0.70792	0.98	W	TUO	426.3	60.1	51.6	70.2	P	F	P	.			
11162	11/26/01	78	3	F	1999	0.70724	0.25	W	TUO*	469.1	67.5	58.9	77.5	P	P	S	68	3.56	0.26	
11174	12/07/01	80	3	F	1999	0.70754	0.83	W	TUO	404.9	56.5	47.9	66.6	P	F	P	61	3.15	0.21	
11192	11/23/01	74	3	F	1999	0.70772	0.94	W	TUO	478.8	69.1	60.5	79.2	P	P	S	74	3.02	0.28	
11209	11/21/01	97	3	M	1999	0.70771	0.94	W	TUO	551.4	81.5	72.9	91.6	S	P	S	85	3.72	0.31	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

11213	11/21/01	83	3	F	1999	0.70771	0.94	W	TUO	483.5	69.9	61.3	80.0	P	P	S	.			
11217	11/21/01	40.5	3	M	1999	0.70764	0.90	W	TUO	447.2	63.7	55.1	73.8	P	P	P	84	2.59	0.23	
14499	11/04/02	93	4	F	1999	0.70814	0.83	W	TUO	542.7	80.0	71.5	90.1	S	P	S	92	2.99	0.21	
14568	11/05/02	107	4	M	1999	0.70761	0.89	W	TUO	591.8	88.4	79.9	98.5	S	S	S	82	3.39	0.25	
14621	11/12/02	104	4	M	1999	0.70776	0.95	W	TUO	538.8	79.4	70.8	89.4	S	P	S	88	2.84	0.25	
14623	11/12/02	85	4	F	1999	0.70782	0.97	W	TUO	492.8	71.5	62.9	81.6	P	P	S	90	2.96	0.20	
14627	11/12/02	97	4	F	1999	0.70754	0.83	W	TUO	437.0	62.0	53.4	72.0	P	F	P	.			
14635	11/12/02	101	4	M	1999	0.70777	0.96	W	TUO	510.0	74.4	65.9	84.5	P	P	S	74	3.37	0.20	
14647	11/12/02	96	4	F	1999	0.70805	0.95	W	TUO	466.8	67.1	58.5	77.1	P	P	S	.			
14669	11/12/02	104	4	M	1999	0.70761	0.89	W	TUO	475.1	68.5	59.9	78.6	P	P	S	80	3.09	0.22	
14687	11/12/02	91	4	F	1999	0.70738	0.56	W	TUO	510.5	74.5	65.9	84.6	P	P	S	81	3.57	0.27	
14693	11/12/02	99	4	M	1999	0.70751	0.80	W	TUO	521.6	76.4	67.8	86.5	S	P	S	84	3.04	0.19	
14716	11/13/02	97	4	M	1999	0.70757	0.85	W	TUO	522.6	76.6	68.0	86.7	S	P	S	80	3.05	0.25	
14729	11/13/02	96	4	M	1999	0.70726	0.28	W	TUO*	462.6	66.3	57.8	76.4	P	P	S	69	3.08	0.21	
14759	11/14/02	101	4	M	1999	0.70766	0.91	W	TUO	508.1	74.1	65.5	84.2	P	P	S	94	2.95	0.24	
14774	11/14/02	86	4	F	1999	0.70786	0.97	W	TUO	483.1	69.9	61.3	79.9	P	P	S	.			
14804	11/14/02	93	4	F	1999	0.70768	0.93	W	TUO	488.6	70.8	62.2	80.9	P	P	S	78	3.07	0.17	
14824	11/15/02	98	4	M	1999	0.70733	0.44	W	TUO*	517.4	75.7	67.1	85.8	S	P	S	*Inconclusive natal assignment*			
14850	11/18/02	92	4	F	1999	0.70722	0.19	W	TUO*	465.4	66.8	58.2	76.9	P	P	S	*Inconclusive natal assignment*			
14884	11/18/02	89	4	F	1999	0.70749	0.77	W	TUO	499.0	72.6	64.0	82.6	P	P	S	.			
14889	11/18/02	88	4	M	1999	0.70754	0.82	W	TUO	508.5	74.2	65.6	84.3	P	P	S	80	3.11	0.22	
14892	11/18/02	100	4	M	1999	0.70725	0.26	W	TUO*	471.9	67.9	59.4	78.0	P	P	S	*Inconclusive natal assignment*			
14904	11/18/02	100	4	M	1999	0.70745	0.70	W	TUO	480.2	69.4	60.8	79.4	P	P	S	61	3.65	0.25	
14919	11/18/02	88	4	F	1999	0.70729	0.35	W	TUO*	409.7	57.3	48.7	67.4	P	F	P	*Inconclusive natal assignment*			
14953	11/19/02	103	4	M	1999	0.70787	0.97	W	TUO	489.5	71.0	62.4	81.0	P	P	S	92	2.91	0.18	
14955	11/19/02	94	4	F	1999	0.70813	0.84	W	TUO	527.1	77.4	68.8	87.4	S	P	S	96	3.28	0.25	
14976	11/19/02	102	4	M	1999	0.70765	0.91	W	TUO	496.3	72.1	63.5	82.2	P	P	S	94	3.21	0.26	
14999	11/20/02	104	4	M	1999	0.70726	0.27	W	TUO*	489.5	71.0	62.4	81.0	P	P	S	67	3.88	0.22	
15001	11/20/02	101	4	M	1999	0.70787	0.97	W	TUO	431.7	61.1	52.5	71.1	P	F	P	74	2.92	0.25	
15052	11/20/02	105	4	M	1999	0.70776	0.95	W	TUO	557.9	82.6	74.1	92.7	S	P	S	.			
15064	11/20/02	98	4	M	1999	0.70775	0.95	W	TUO	415.1	58.2	49.6	68.3	P	F	P	49	3.21	0.25	
15097	11/21/02	104	4	M	1999	0.70774	0.95	W	TUO	539.3	79.5	70.9	89.5	S	P	S	101	3.22	0.26	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

15146	11/24/02	107	4	M	1999	0.70721	0.19	W	TUO*	451.0	64.4	55.8	74.4	P	P	P	*Inconclusive natal assignment*			
15150	11/24/02	108	4	M	1999	0.70761	0.89	W	TUO	436.4	61.9	53.3	71.9	P	F	P	64	3.34	0.28	
15165	11/24/02	100	4	M	1999	0.70726	0.27	W	TUO*	406.9	56.8	48.2	66.9	P	F	P	*Inconclusive natal assignment*			
19679	11/15/01	78	3	F	1999	0.70754	0.83	W	TUO	514.2	75.2	66.6	85.2	S	P	S	98	3.26	0.26	
19686	11/15/01	81	3	F	1999	0.70780	0.96	W	TUO	518.4	75.9	67.3	85.9	S	P	S	96	3.40	0.22	
19688	11/15/01	72.5	3	F	1999	0.70772	0.94	W	TUO	554.6	82.1	73.5	92.1	S	P	S	.			
19705	11/15/01	76	3	F	1999	0.70729	0.35	W	TUO*	373.8	51.2	42.6	61.2	F	F	P	*Inconclusive natal assignment*			
19722	11/19/01	87	3	F	1999	0.70733	0.44	W	TUO*	553.2	81.8	73.3	91.9	S	P	S	*Inconclusive natal assignment*			
19775	11/28/01	83	3	F	1999	0.70736	0.52	W	TUO	509.5	74.4	65.8	84.4	P	P	S	81	3.44	0.25	
19779	11/28/01	70	3	F	1999	0.70760	0.88	W	TUO	477.0	68.8	60.2	78.9	P	P	S	65	3.63	0.22	
19782	11/28/01	76	3	M	1999	0.70779	0.96	W	TUO	514.6	75.2	66.7	85.3	S	P	S	89	3.03	0.19	
19786	11/28/01	85	3	M	1999	0.70746	0.73	W	TUO	549.0	81.1	72.5	91.2	S	P	S	101	3.30	0.26	
19791	11/28/01	79	3	F	1999	0.70735	0.49	W	TUO*	461.2	66.1	57.5	76.2	P	P	S	*Inconclusive natal assignment*			
19792	11/28/01	76	3	F	1999	0.70814	0.83	W	TUO	486.8	70.5	61.9	80.5	P	P	S	82	3.78	0.25	
19797	12/03/01	84	3	F	1999	0.70735	0.48	W	TUO*	458.1	65.6	57.0	75.6	P	P	S	*Inconclusive natal assignment*			
19816	12/03/01	81	3	M	1999	0.70770	0.93	W	TUO	632.3	95.4	86.8	105.4	S	S	S	.			
19836	12/03/01	88	3	M	1999	0.70726	0.29	W	TUO*	451.4	64.4	55.8	74.5	P	P	P	*Inconclusive natal assignment*			
19841	12/03/01	74	3	F	1999	0.70724	0.23	W	TUO*	457.9	65.5	57.0	75.6	P	P	S	*Inconclusive natal assignment*			
19845	12/04/01	87	3	M	1999	0.70788	0.97	W	TUO	514.2	75.2	66.6	85.2	S	P	S	93	2.96	0.20	
19855	12/04/01	85	3	M	1999	0.70768	0.92	W	TUO	441.7	62.8	54.2	72.8	P	F	P	61	3.15	0.32	
19861	12/04/01	74	3	F	1999	0.70775	0.95	W	TUO	494.2	71.7	63.2	81.8	P	P	S	80	3.13	0.22	
19866	12/04/01	86	3	M	1999	0.70724	0.23	W	TUO*	467.7	67.2	58.6	77.3	P	P	S	63	3.24	0.27	
19868	12/04/01	74	3	F	1999	0.70736	0.51	W	TUO	583.5	87.0	78.4	97.1	S	S	S	97	3.86	0.29	
19874	12/10/01	75	3	F	1999	0.70756	0.84	W	TUO	372.4	50.9	42.3	61.0	F	F	P	61	3.21	0.26	
19876	12/10/01	71	3	M	1999	0.70765	0.91	W	TUO	507.2	74.0	65.4	84.0	P	P	S	79	3.48	0.20	
11055	11/20/01	56	2	M	2000	0.70770	0.93	W	TUO	354.0	47.8	39.2	57.8	F	F	P	.			
11063	11/20/01	58	2	M	2000	0.70763	0.90	W	TUO	524.0	76.8	68.3	86.9	S	P	S	81	3.13	0.22	
11076	11/20/01	81	2	F	2000	0.70807	0.93	W	TUO	509.0	74.3	65.7	84.3	P	P	S	98	2.98	0.23	
11083	11/20/01	59	2	M	2000	0.70742	0.65	W	TUO	419.0	58.9	50.3	69.0	P	F	P	49	3.28	0.23	
11111	11/08/01	54.5	2	F	2000	0.70775	0.95	W	TUO	477.0	68.8	60.2	78.9	P	P	S	81	3.00	0.23	
11133	11/26/01	59	2	F	2000	0.70797	0.97	W	TUO	472.0	68.0	59.4	78.0	P	P	S	.			
11167	11/26/01	60	2	F	2000	0.70752	0.81	W	TUO	451.0	64.4	55.8	74.4	P	P	P	61	2.99	0.29	
11212	11/21/01	65.5	2	F	2000	0.70760	0.88	W	TUO	465.0	66.8	58.2	76.8	P	P	S	60	3.38	0.26	

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11215	11/21/01	60	2	F	2000	0.70795	0.98	W	TUO	508.0	74.1	65.5	84.2	P	P	S	69	3.84	0.26	
11220	10/31/01	62	2	F	2000	0.70789	0.97	W	TUO	548.0	80.9	72.4	91.0	S	P	S	84	3.37	0.30	
11223	10/31/01	54	2	M	2000	0.70784	0.97	W	TUO	494.0	71.7	63.1	81.8	P	P	S	77	3.05	0.21	
11228	10/31/01	60	2	M	2000	0.70778	0.96	W	TUO	407.0	56.8	48.2	66.9	P	F	P	66	3.11	0.19	
14528	11/04/02	95	3	M	2000	0.70813	0.85	W	TUO	513.0	75.0	66.4	85.0	P	P	S	84	2.93	0.24	
14539	11/04/02	72	3	F	2000	0.70789	0.97	W	TUO	451.0	64.4	55.8	74.4	P	P	P	69	2.97	0.19	
14540	11/04/02	69	3	F	2000	0.70777	0.96	INC	TUO*	438.0	62.1	53.6	72.2	P	F	P	.			Unreadable, so cannot assign natal location or do ageing
14544	11/05/02	78	3	F	2000	0.70782	0.96	W	TUO	429.0	60.6	52.0	70.7	P	F	P	48	2.90	0.22	
14545	11/05/02	92	3	M	2000	0.70792	0.98	W	TUO	404.0	56.3	47.7	66.4	P	F	P	.			
14548	11/05/02	72	3	F	2000	0.70801	0.97	W	TUO	574.0	85.4	76.8	95.5	S	S	S	.			
14550	11/05/02	80	3	M	2000	0.70789	0.97	W	TUO	387.0	53.4	44.8	63.5	F	F	P	62	2.75	0.25	
14556	11/05/02	73	3	F	2000	0.70742	0.64	W	TUO	433.0	61.3	52.7	71.4	P	F	P	71	3.15	0.26	
14559	11/05/02	89	3	M	2000	0.70768	0.92	W	TUO	382.0	52.6	44.0	62.6	F	F	P	.			
14560	11/05/02	78	3	F	2000	0.70756	0.85	W	TUO	434.0	61.5	52.9	71.5	P	F	P	69	2.96	0.22	
14566	11/05/02	79	3	F	2000	0.70786	0.97	W	TUO	431.0	60.9	52.4	71.0	P	F	P	.			
14571	11/05/02	97	3	M	2000	0.70763	0.90	W	TUO	502.0	73.1	64.5	83.1	P	P	S	77	2.91	0.24	
14575	11/05/02	73	3	F	2000	0.70795	0.98	W	TUO	438.0	62.1	53.6	72.2	P	F	P	72	3.07	0.22	
14578	11/05/02	73	3	F	2000	0.70745	0.71	W	TUO	411.0	57.5	48.9	67.6	P	F	P	.			
14579	11/05/02	93	3	M	2000	0.70742	0.65	W	TUO	413.0	57.9	49.3	67.9	P	F	P	60	3.41	0.30	
14584	11/05/02	81	.	M	2000	0.70772	0.94	W	TUO	464.0	66.6	58.0	76.7	P	P	S	.			
14587	11/05/02	80	3	F	2000	0.70780	0.96	W	TUO	508.0	74.1	65.5	84.2	P	P	S	.			
14596	11/05/02	80	3	F	2000	0.70800	0.97	W	TUO	516.0	75.5	66.9	85.5	S	P	S	99	2.97	0.22	
14597	11/05/02	91	3	M	2000	0.70756	0.85	W	TUO	409.0	57.2	48.6	67.2	P	F	P	57	3.05	0.29	
14600	11/05/02	75	3	F	2000	0.70774	0.95	W	TUO	383.0	52.7	44.1	62.8	F	F	P	.			
14616	11/12/02	94	3	M	2000	0.70768	0.92	W	TUO	344.0	46.1	37.5	56.1	F	F	P	18	2.56	0.13	
14626	11/12/02	91	3	M	2000	0.70735	0.50	W	TUO	461.0	66.1	57.5	76.1	P	P	S	.			
14629	11/12/02	74	3	F	2000	0.70772	0.94	W	TUO	486.0	70.3	61.8	80.4	P	P	S	.			
14661	11/12/02	94	3	M	2000	0.70761	0.89	W	TUO	412.0	57.7	49.1	67.8	P	F	P	73	2.64	0.31	
14668	11/12/02	90	3	M	2000	0.70785	0.97	W	TUO	466.0	66.9	58.3	77.0	P	P	S	75	2.93	0.21	
14673	11/12/02	90	3	M	2000	0.70770	0.93	W	TUO	447.0	63.7	55.1	73.7	P	P	P	61	3.28	0.22	
14689	11/12/02	93	3	M	2000	0.70803	0.96	W	TUO	465.0	66.8	58.2	76.8	P	P	S	87	3.34	0.23	Microstructure ran out 13um before last natal spot (inferred 4

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																				increments)
14701	11/13/02	93	3	M	2000	0.70803	0.96	W	TUO	456.0	65.2	56.6	75.3	P	P	S	60	2.32	0.25	Strange profile (used same distance for natal and FW exit)
14721	11/13/02	76	3	F	2000	0.70804	0.95	W	TUO	453.0	64.7	56.1	74.8	P	P	P	75	3.05	0.25	
14735	11/13/02	92	3	M	2000	0.70763	0.90	W	TUO	367.0	50.0	41.4	60.1	F	F	P	44	3.19	0.17	
14743	11/13/02	76	3	F	2000	0.70802	0.96	W	TUO	479.0	69.1	60.6	79.2	P	P	S	89	2.89	0.22	
14749	11/14/02	80	3	F	2000	0.70773	0.94	W	TUO	372.0	50.9	42.3	60.9	F	F	P	45	2.86	0.17	
14753	11/14/02	84	3	M	2000	0.70774	0.95	W	TUO	393.0	54.4	45.9	64.5	F	F	P	64	3.10	0.20	
14769	11/14/02	81	3	F	2000	0.70757	0.85	W	TUO	378.0	51.9	43.3	61.9	F	F	P	51	3.19	0.27	
14783	11/14/02	89	3	M	2000	0.70793	0.98	W	TUO	496.0	72.1	63.5	82.1	P	P	S	77	3.43	0.22	
14785	11/14/02	95	3	M	2000	0.70766	0.91	W	TUO	447.0	63.7	55.1	73.7	P	P	P	.			
14786	11/14/02	76	3	F	2000	0.70781	0.96	W	TUO	421.0	59.2	50.6	69.3	P	F	P	51	3.57	0.20	
14813	11/14/02	86	3	M	2000	0.70799	0.97	W	TUO	430.0	60.8	52.2	70.8	P	F	P	65	3.08	0.24	
14815	11/15/02	82	3	F	2000	0.70761	0.89	W	TUO	438.0	62.1	53.6	72.2	P	F	P	.			
14858	11/18/02	104	3	M	2000	0.70740	0.61	W	TUO	464.2	66.6	58.0	76.7	P	P	S	73	3.23	0.29	
14880	11/18/02	80	3	M	2000	0.70727	0.31	W	TUO*	409.0	57.2	48.6	67.2	P	F	P	48	3.57	0.28	
14907	11/18/02	74	3	F	2000	0.70773	0.95	W	TUO	475.0	68.5	59.9	78.5	P	P	S	71	3.08	0.22	
14921	11/18/02	93	3.5	F	2000	0.70780	0.96	W	TUO	377.0	51.7	43.1	61.8	F	F	P	28	3.08	0.21	
14929	11/18/02	91	3	M	2000	0.70764	0.90	W	TUO	454.0	64.9	56.3	74.9	P	P	P	67	3.37	0.27	
14975	11/19/02	102	3	M	2000	0.70743	0.67	W	TUO	392.0	54.3	45.7	64.3	F	F	P	.			
15091	11/21/02	100	3	F	2000	0.70792	0.98	W	TUO	497.0	72.2	63.6	82.3	P	P	S	60	3.52	0.23	
15113	11/21/02	100	3	M	2000	0.70763	0.90	W	TUO	407.0	56.8	48.2	66.9	P	F	P	.			
15133	11/24/02	103	3	M	2000	0.70754	0.83	W	TUO	428.0	60.4	51.8	70.5	P	F	P	67	3.06	0.25	
15193	11/24/02	101	3	M	2000	0.70773	0.94	W	TUO	508.0	74.1	65.5	84.2	P	P	S	79	3.11	0.25	
15243	12/02/02	91	3	M	2000	0.70777	0.95	W	TUO	414.0	58.0	49.4	68.1	P	F	P	71	3.02	0.27	
19681	11/15/01	65	2	M	2000	0.70759	0.87	W	TUO	383.0	52.7	44.1	62.8	F	F	P	.			
19695	11/19/01	59.5	2	M	2000	0.70759	0.88	W	TUO	488.0	70.7	62.1	80.8	P	P	S	74	3.09	0.26	
19813	12/03/01	48	2	M	2000	0.70789	0.97	W	TUO	503.0	73.3	64.7	83.3	P	P	S	133	3.11	0.26	
19831	12/03/01	57	2	F	2000	0.70779	0.96	W	TUO	386.0	53.2	44.7	63.3	F	F	P	48	3.29	0.24	
19853	12/04/01	60	2	F	2000	0.70781	0.96	W	TUO	517.0	75.6	67.1	85.7	S	P	S	90	3.14	0.29	
19858	12/04/01	58	2	F	2000	0.70761	0.89	W	TUO	437.0	62.0	53.4	72.0	P	F	P	.			
17628	11/14/05	91	3	M	2003	0.70730	0.37	W	TUO*	459.8	65.9	57.3	75.9	P	P	S	70	3.67	0.26	
17631	11/14/05	84	3	F	2003	0.70751	0.80	W	TUO	554.2	82.0	73.4	92.1	S	P	S	94	3.80	0.25	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

17634	11/14/05	81	3	F	2003	0.70772	0.94	W	TUO	475.1	68.5	59.9	78.6	P	P	S	94	2.57	0.22	
17637	11/16/05	92	3	M	2003	0.70726	0.27	W	TUO*	381.2	52.4	43.8	62.5	F	F	P	*Inconclusive natal assignment*			
17638	11/16/05	76	3	F	2003	0.70731	0.41	W	TUO*	536.5	79.0	70.4	89.0	S	P	S	81	3.60	0.24	
17645	11/21/05	75	3	F	2003	0.70747	0.74	W	TUO	557.4	82.6	74.0	92.6	S	P	S	.			
17651	11/21/05	88	3	M	2003	0.70745	0.71	W	TUO	538.8	79.4	70.8	89.4	S	P	S	76	3.98	0.22	
17654	11/21/05	73	3	F	2003	0.70757	0.86	W	TUO	309.6	40.2	31.6	50.3	F	F	F	57	2.79	0.26	
17666	11/28/05	73	3	F	2003	0.70753	0.82	W	TUO	485.8	70.3	61.7	80.4	P	P	S	88	3.09	0.20	
17667	11/28/05	75	3	F	2003	0.70744	0.69	W	TUO	493.7	71.7	63.1	81.7	P	P	S	97	3.34	0.27	
17669	11/28/05	72	3	F	2003	0.70743	0.66	W	TUO	504.9	73.6	65.0	83.6	P	P	S	.			
17672	11/28/05	79	3	F	2003	0.70756	0.85	INC	TUO*	n/a (vaterite)	n/a	n/a	n/a	n/a	n/a	n/a	.			Otolith was vateritic during natal rearing (so no HvW assignment or exit age/distance)
17673	11/28/05	71	3	F	2003	0.70745	0.70	W	TUO	450.5	64.3	55.7	74.3	P	P	P	.			
17679	11/28/05	85	3	M	2003	0.70729	0.34	W	TUO*	452.8	64.7	56.1	74.7	P	P	P	53	3.86	0.22	
17680	11/28/05	75	3	F	2003	0.70777	0.96	W	TUO	507.7	74.1	65.5	84.1	P	P	S	85	2.92	0.26	Microstructure ran out 54um before last natal spot (inferred 18 increments)
17681	11/28/05	72	3	F	2003	0.70763	0.90	W	TUO	441.7	62.8	54.2	72.8	P	F	P	.			
17685	11/28/05	61	3	M	2003	0.70727	0.30	W	TUO*	419.8	59.0	50.4	69.1	P	F	P	*Inconclusive natal assignment*			
17690	11/28/05	83	3	M	2003	0.70754	0.83	W	TUO	565.8	84.0	75.4	94.1	S	S	S	102	3.61	0.28	
17692	11/29/05	85	3	F	2003	0.70759	0.87	W	TUO	432.4	61.2	52.6	71.2	P	F	P	46	4.20	0.19	
17703	12/06/05	75	3	F	2003	0.70751	0.79	W	TUO	599.2	89.7	81.1	99.8	S	S	S	108	3.33	0.19	
17712	12/06/05	90	3	M	2003	0.70734	0.46	W	TUO*	456.5	65.3	56.7	75.4	P	P	S	*Inconclusive natal assignment*			
17713	12/06/05	76	3	F	2003	0.70755	0.84	W	TUO	446.3	63.6	55.0	73.6	P	F	P	66	3.63	0.20	
17716	12/06/05	82	3	F	2003	0.70744	0.69	W	TUO	514.6	75.2	66.7	85.3	S	P	S	.			
17718	12/07/05	79	3	F	2003	0.70751	0.79	W	TUO	530.9	78.0	69.4	88.1	S	P	S	.			
17729	12/12/05	92	3	M	2003	0.70754	0.82	W	TUO	481.6	69.6	61.0	79.7	P	P	S	.			
17740	12/12/05	79	3	F	2003	0.70738	0.56	W	TUO	453.7	64.8	56.2	74.9	P	P	P	.			
17742	12/12/05	75	3	F	2003	0.70743	0.66	W	TUO	416.6	58.5	49.9	68.5	P	F	P	65	3.09	0.21	
17746	12/12/05	72	3	F	2003	0.70762	0.89	W	TUO	599.7	89.8	81.2	99.9	S	S	S	.			
17751	12/12/05	85	3	F	2003	0.70755	0.84	W	TUO	504.9	73.6	65.0	83.6	P	P	S	93	3.49	0.21	
17753	12/12/05	81	3	M	2003	0.70750	0.79	W	TUO	478.8	69.1	60.5	79.2	P	P	S	65	3.50	0.26	
17758	12/12/05	84	3	F	2003	0.70718	0.14	W	TUO*	501.2	72.9	64.4	83.0	P	P	S	76	3.59	0.21	

Appendix 1A Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the Tuolumne River (natal exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable. The life stage at natal river exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

17759	12/12/05	70	3	F	2003	0.70765	0.91	W	TUO	487.2	70.6	62.0	80.6	P	P	S	.			
17763	12/12/05	79	3	F	2003	0.70744	0.70	W	TUO	461.6	66.2	57.6	76.2	P	P	S	74	3.59	0.19	
18144	12/05/06	86	4	F	2003	0.70726	0.29	W	TUO*	502.1	73.1	64.5	83.2	P	P	S	78	3.37	0.22	
18150	12/11/06	84	4	F	2003	0.70741	0.62	W	TUO	444.9	63.3	54.7	73.4	P	F	P	65	3.30	0.17	
24120	11/07/11	76	3	F	2009	0.70777	0.73	W	TUO	451.3	64.4	55.8	74.5	P	P	P	66	3.29	0.23	
24176	11/14/11	81	3	F	2009	0.70781	0.77	W	TUO	542.0	79.9	71.3	90.0	S	P	S	76	3.78	0.24	
24178	11/14/11	67	3	M	2009	0.70778	0.74	W	TUO	559.0	81.0	72.4	91.0	S	P	S	109	3.19	0.23	
24238	11/21/11	70	3	F	2009	0.70773	0.69	W	TUO	508.2	74.1	65.5	84.2	P	P	S	.			
24283	11/23/11	83	3	M	2009	0.70734	0.16	W	TUO*	479.7	69.3	60.7	79.3	P	P	S	*Inconclusive natal assignment*			
24292	11/28/11	95	3	M	2009	0.70730	0.12	W	TUO*	555.9	82.3	73.7	92.4	S	P	S	*Inconclusive natal assignment*			
26012	11/13/12	84	4	F	2009	0.70780	0.76	W	TUO	546.1	80.6	72.0	90.7	S	P	S	101	3.16	0.23	

[1] Assignments using isotope-based discriminant function analysis and reference samples from existing or ongoing projects ([1], [2], P. Weber, A. Sturrock, unpub)
[2] Hatchery vs. wild assignment using microstructure-based discriminant function analysis and existing reference samples, after [3].
[3] Size-defined life stage designations (fry: <55mm, parr: >55mm to <75mm, smolt: >75mm), after [4].

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

						Natal Sr ratio				FW EXIT	Predicted FL at natal exit (mm)			Predicted life stage at natal exit [3]				Increment width (um)		
Sample ID	Capture date	Capture FL (cm)	Scale age	Sex	Outmi-gration year	Mean natal value	Prob to TUO [1]	H vs. W [2]	Natal location	Otolith distance (um)	FL	Lower 95% CI	Upper 95% CI	Life stage	Lower 95% CI	Upper 95% CI	Increment number (days)	Mean	CV	Notes
4175	10/10/00	98	3	M	1998	0.70799	0.97	W	TUO	603.6	90.4	81.9	100.5	S	S	S	4	3.1	0.2	
4176	10/10/00	91	3	M	1998	0.70774	0.95	W	TUO	604.3	90.6	82.0	100.6	S	S	S	22	3.1	0.2	
4182	10/17/00	76	3	M	1998	0.70803	0.96	W	TUO	578.3	86.1	77.5	96.2	S	S	S	11	2.8	0.3	
4183	10/17/00	90	3	M	1998	0.70797	0.97	W	TUO	514.7	75.3	66.7	85.3	S	P	S	16	3.7	0.2	
4185	10/17/00	84	3	F	1998	0.70728	0.64	W	TUO	585.4	87.3	78.7	97.4	S	S	S	30	2.6	0.2	
4189	10/24/00	90	3	F	1998	0.70806	0.94	W	TUO	496.4	72.1	63.5	82.2	P	P	S	11	2.9	0.3	
4192	10/24/00	87.5	3	F	1998	0.70807	0.93	W	TUO	517.3	75.7	67.1	85.8	S	P	S	5	5.3	0.3	
4196	10/24/00	67.9	3	F	1998	0.70800	0.97	W	TUO	524.7	77.0	68.4	87.0	S	P	S	n/a	n/a	n/a	
4197	10/24/00	78.6	3	F	1998	0.70740	0.61	W	TUO	531.8	78.2	69.6	88.2	S	P	S	23	3.8	0.2	
4200	10/24/00	68.6	3	F	1998	0.70760	0.88	W	TUO	611.4	91.8	83.2	101.9	S	S	S	9	3.4	0.2	
4210	10/24/00	88.3	3	M	1998	0.70764	0.91	W	TUO	511.3	74.7	66.1	84.7	P	P	S	8	3.9	0.2	
4211	10/24/00	72	3	F	1998	0.70783	0.97	W	TUO	625.4	94.2	85.6	104.2	S	S	S	.			
4212	10/24/00	78.1	3	M	1998	0.70765	0.91	W	TUO	568.9	84.5	75.9	94.6	S	S	S	13	2.8	0.3	
4215	10/25/00	79	3	F	1998	0.70802	0.96	W	TUO	620.1	93.3	84.7	103.3	S	S	S	18	3.5	0.2	
4226	10/25/00	80	3	F	1998	0.70770	0.93	W	TUO	479.9	69.3	60.7	79.4	P	P	S	6	4.1	0.2	
4232	10/25/00	88.5	3	M	1998	0.70821	0.53	W	TUO	540.3	79.6	71.0	89.7	S	P	S	.			
4233	10/25/00	72	3	F	1998	0.70795	0.98	W	TUO	504.7	73.5	64.9	83.6	P	P	S	18	2.5	0.2	
4234	10/25/00	77	3	F	1998	0.70823	0.45	W	TUO*	607.6	91.1	82.6	101.2	S	S	S	*Inconclusive natal assignment*			
4240	10/26/00	80	3	F	1998	0.70726	0.28	W	TUO*	484.4	70.1	61.5	80.1	P	P	S	*Inconclusive natal assignment*			
4249	10/30/00	80	3	F	1998	0.70737	0.54	W	TUO	520.5	76.2	67.6	86.3	S	P	S	.			
4253	10/30/00	80	3	M	1998	0.70810	0.90	W	TUO	595.9	89.1	80.6	99.2	S	S	S	16	2.8	0.3	
4266	10/30/00	77	3	F	1998	0.70740	0.60	W	TUO	508.6	74.2	65.6	84.3	P	P	S	19	2.7	0.2	
4267	10/30/00	75	3	F	1998	0.70812	0.86	W	TUO	534.2	78.6	70.0	88.7	S	P	S	.			
4269	10/30/00	80	3	F	1998	0.70732	0.43	W	TUO*	662.8	100.6	92.0	110.6	S	S	S	*Inconclusive natal assignment*			
4275	10/31/00	79	3	F	1998	0.70721	0.18	W	TUO*	444.6	63.3	54.7	73.3	P	F	P	*Inconclusive natal assignment*			
4278	10/31/00	83	3	F	1998	0.70802	0.96	W	TUO	515.7	75.4	66.8	85.5	S	P	S	.			
4279	10/31/00	87.5	3	F	1998	0.70798	0.97	W	TUO	596.6	89.3	80.7	99.3	S	S	S	7	3.0	0.2	
4281	10/31/00	91	3	M	1998	0.70728	0.31	W	TUO*	523.6	76.8	68.2	86.8	S	P	S	*Inconclusive natal assignment*			
4292	10/31/00	74	3	F	1998	0.70733	0.44	W	TUO*	608.5	91.3	82.7	101.4	S	S	S	*Inconclusive natal assignment*			
4294	10/31/00	86	3	F	1998	0.70800	0.97	W	TUO	586.2	87.5	78.9	97.6	S	S	S	.			

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4295	10/31/00	72	3	F	1998	0.70816	0.77	W	TUO	518.5	75.9	67.3	86.0	S	P	S	10	2.8	0.2	
4297	11/01/00	74	3	F	1998	0.70805	0.95	W	TUO	644.1	97.4	88.8	107.4	S	S	S	13	3.3	0.2	
4299	11/06/00	81	3	F	1998	0.70801	0.97	W	TUO	555.3	82.2	73.6	92.3	S	P	S	.			
4300	11/06/00	96	3	M	1998	0.70735	0.50	W	TUO*	520.0	76.2	67.6	86.2	S	P	S	*Inconclusive natal assignment*			
4306	11/06/00	85	3	F	1998	0.70801	0.97	W	TUO	576.1	85.7	77.2	95.8	S	S	S	18	3.1	0.2	
4309	11/06/00	84	3	F	1998	0.70807	0.93	W	TUO	453.7	64.8	56.2	74.9	P	P	P	.			
4311	11/06/00	74	3	F	1998	0.70752	0.81	W	TUO	503.0	73.3	64.7	83.3	P	P	S	18	3.2	0.2	
4316	11/06/00	81	3	F	1998	0.70738	0.55	W	TUO	428.0	60.4	51.8	70.5	P	F	P	.			
4317	11/06/00	79	3	F	1998	0.70786	0.97	W	TUO	520.8	76.3	67.7	86.4	S	P	S	n/a	n/a	n/a	Microstructure ran out before fish left natal river
4321	11/06/00	70	3	F	1998	0.70742	0.65	W	TUO	592.5	88.6	80.0	98.6	S	S	S	29	3.9	0.3	
4331	11/07/00	86	3	M	1998	0.70798	0.97	W	TUO	604.8	90.7	82.1	100.7	S	S	S	10	2.3	0.2	
4334	11/07/00	85	3	F	1998	0.70739	0.59	W	TUO	439.7	62.4	53.8	72.5	P	F	P	11	3.4	0.1	
4337	11/07/00	74	3	F	1998	0.70733	0.45	W	TUO*	601.7	90.1	81.5	100.2	S	S	S	*Inconclusive natal assignment*			
4340	11/07/00	75.5	3	F	1998	0.70783	0.97	W	TUO	531.7	78.2	69.6	88.2	S	P	S	.			
4343	11/07/00	81	3	F	1998	0.70768	0.92	W	TUO	545.2	80.5	71.9	90.5	S	P	S	11	3.5	0.2	
4352	11/07/00	73	3	F	1998	0.70788	0.97	W	TUO	588.8	87.9	79.3	98.0	S	S	S	26	2.5	0.3	
4360	11/08/00	76.5	3	F	1998	0.70818	0.67	W	TUO	596.3	89.2	80.6	99.3	S	S	S	.			
4376	11/09/00	85	3	F	1998	0.70733	0.46	W	TUO*	621.8	93.6	85.0	103.6	S	S	S	*Inconclusive natal assignment*			
4378	11/09/00	88	3	M	1998	0.70728	0.33	W	TUO*	647.5	98.0	89.4	108.0	S	S	S	*Inconclusive natal assignment*			
4381	11/13/00	90	3	M	1998	0.70816	0.75	W	TUO	483.0	69.8	61.2	79.9	P	P	S	10	3.1	0.2	
4383	11/13/00	79	3	M	1998	0.70756	0.85	W	TUO	567.9	84.3	75.8	94.4	S	S	S	.			
4384	11/13/00	80	3	F	1998	0.70786	0.97	W	TUO	563.9	83.7	75.1	93.7	S	S	S	20	3.5	0.3	
4397	11/13/00	67	3	F	1998	0.70819	0.66	W	TUO	515.4	75.4	66.8	85.4	S	P	S	14	2.9	0.3	
4403	11/14/00	77	3	F	1998	0.70808	0.92	W	TUO	538.8	79.4	70.8	89.4	S	P	S	10	3.3	0.2	
4414	11/14/00	81	3	F	1998	0.70749	0.77	W	TUO	507.4	74.0	65.4	84.1	P	P	S	.			
4418	11/14/00	86	3	F	1998	0.70742	0.66	W	TUO	520.4	76.2	67.6	86.3	S	P	S	17	4.7	0.2	
4424	11/14/00	77	3	F	1998	0.70783	0.97	W	TUO	591.3	88.3	79.8	98.4	S	S	S	10	3.2	0.2	
4441	11/20/00	72	3	F	1998	0.70823	0.45	W	TUO*	531.0	78.0	69.5	88.1	S	P	S	*Inconclusive natal assignment*			
4442	11/20/00	95	3	M	1998	0.70771	0.94	W	TUO	631.7	95.3	86.7	105.3	S	S	S	26	3.1	0.4	
4443	11/20/00	100	3	M	1998	0.70735	0.50	W	TUO*	541.5	79.8	71.3	89.9	S	P	S	*Inconclusive natal assignment*			
4450	11/20/00	82	3	F	1998	0.70817	0.73	W	TUO	447.6	63.8	55.2	73.8	P	P	P	0	n/a	n/a	Strange profile (used same distance for natal and FW exit)

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

4451	11/20/00	92	3	M	1998	0.70817	0.72	W	TUO	507.3	74.0	65.4	84.1	P	P	S	25	2.7	0.2	
4455	11/20/00	74	3	F	1998	0.70769	0.93	W	TUO	533.3	78.4	69.9	88.5	S	P	S	12	3.6	0.3	
4458	11/21/00	80	3	F	1998	0.70792	0.98	W	TUO	591.4	88.4	79.8	98.4	S	S	S	16	3.7	0.2	
4476	11/22/00	100	3	M	1998	0.70804	0.95	W	TUO	556.0	82.3	73.7	92.4	S	P	S	15	3.1	0.3	
4484	11/27/00	77	3	F	1998	0.70788	0.97	W	TUO	557.1	82.5	73.9	92.6	S	P	S	12	3.5	0.1	
4487	11/27/00	84	3	F	1998	0.70800	0.97	W	TUO	533.6	78.5	69.9	88.6	S	P	S	.			
4504	12/04/00	100	3	M	1998	0.70826	0.30	W	TUO*	517.5	75.7	67.1	85.8	S	P	S	*Inconclusive natal assignment*			
4506	12/04/00	80	3	F	1998	0.70756	0.85	W	TUO	458.0	65.6	57.0	75.6	P	P	S	15	3.7	0.2	
4508	12/04/00	89	3	F	1998	0.70806	0.94	W	TUO	500.1	72.8	64.2	82.8	P	P	S	25	2.7	0.2	
4509	12/04/00	70.5	3	F	1998	0.70812	0.86	W	TUO	520.7	76.3	67.7	86.4	S	P	S	14	4.0	0.2	
4510	12/05/00	77	3	F	1998	0.70776	0.95	W	TUO	538.9	79.4	70.8	89.5	S	P	S	27	4.3	0.2	
4514	12/05/00	78	3	F	1998	0.70794	0.98	W	TUO	488.2	70.7	62.1	80.8	P	P	S	.			
4515	12/05/00	77	3	F	1998	0.70815	0.80	W	TUO	545.4	80.5	71.9	90.6	S	P	S	9	3.1	0.1	
4516	12/05/00	82	3	F	1998	0.70818	0.69	W	TUO	497.1	72.2	63.7	82.3	P	P	S	9	3.7	0.2	
4517	12/05/00	88.5	3	F	1998	0.70798	0.97	W	TUO	555.9	82.3	73.7	92.4	S	P	S	16	3.0	0.2	
4518	12/05/00	83	3	F	1998	0.70789	0.97	W	TUO	563.3	83.6	75.0	93.6	S	P	S	15	3.0	0.2	
4521	12/06/00	78.5	3	F	1998	0.70788	0.97	W	TUO	563.3	83.6	75.0	93.6	S	P	S	14	3.2	0.2	
4527	12/11/00	83	3	M	1998	0.70819	0.66	W	TUO	604.6	90.6	82.0	100.7	S	S	S	30	2.9	0.2	
4535	12/19/00	78	3	F	1998	0.70814	0.82	W	TUO	541.6	79.9	71.3	89.9	S	P	S	11	3.5	0.1	
9536	07/07/00	75	3	F	1998	0.70775	0.95	W	TUO	752.4	115.9	107.3	126.0	S	S	S	.			
11015	11/16/01	86.5	4	F	1998	0.70789	0.97	W	TUO	548.6	81.0	72.5	91.1	S	P	S	10	4.3	0.2	
11036	12/11/01	86	4	F	1998	0.70772	0.94	W	TUO	617.6	92.8	84.3	102.9	S	S	S	17	2.6	0.2	
11037	12/11/01	110	4	M	1998	0.70792	0.98	W	TUO	592.2	88.5	79.9	98.6	S	S	S	18	3.6	0.2	
11038	12/11/01	78	4	F	1998	0.70821	0.53	W	TUO	504.6	73.5	64.9	83.6	P	P	S	.			
11040	12/11/01	98	4	F	1998	0.70812	0.86	W	TUO	595.5	89.1	80.5	99.1	S	S	S	18	3.0	0.2	
11056	11/20/01	78	4	F	1998	0.70779	0.96	W	TUO	571.4	85.0	76.4	95.0	S	S	S	19	2.9	0.3	
11064	11/20/01	95	4	M	1998	0.70745	0.71	W	TUO	586.3	87.5	78.9	97.6	S	S	S	10	4.0	0.4	
11072	11/20/01	112	4	M	1998	0.70816	0.75	W	TUO	500.3	72.8	64.2	82.9	P	P	S	9	3.5	0.1	
11085	11/20/01	87	4	F	1998	0.70737	0.55	W	TUO	448.5	63.9	55.4	74.0	P	P	P	.			
11089	11/20/01	104	4	M	1998	0.70816	0.76	W	TUO	509.0	74.3	65.7	84.3	P	P	S	17	2.5	0.3	
11097	11/30/01	82	4	M	1998	0.70743	0.67	W	TUO	573.9	85.4	76.8	95.4	S	S	S	29	2.8	0.3	
11098	11/30/01	87	4	F	1998	0.70807	0.93	W	TUO	553.7	81.9	73.3	92.0	S	P	S	10	3.4	0.1	
11140	11/26/01	88	4	F	1998	0.70769	0.93	W	TUO	526.7	77.3	68.7	87.4	S	P	S	18	3.1	0.2	
11154	11/26/01	87	4	F	1998	0.70794	0.98	W	TUO	568.6	84.5	75.9	94.5	S	S	S	18	2.8	0.1	

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

11176	12/07/01	92.5	4	F	1998	0.70821	0.54	W	TUO	605.9	90.8	82.3	100.9	S	S	S	.			
11177	12/07/01	90	4	F	1998	0.70781	0.96	W	TUO	546.6	80.7	72.1	90.8	S	P	S	.			
11181	12/18/01	87	3	F	1998	0.70819	0.63	W	TUO	730.2	112.1	103.5	122.2	S	S	S	42	2.5	0.2	
11182	12/17/01	99	4	M	1998	0.70798	0.97	W	TUO	417.2	58.6	50.0	68.7	P	F	P	3	3.56	0.2 4	
11190	11/23/01	90	3	M	1998	0.70821	0.53	W	TUO	540.6	79.7	71.1	89.8	S	P	S	18	2.6	0.2	
11216	11/21/01	94	4	F	1998	0.70821	0.54	W	TUO	552.9	81.8	73.2	91.9	S	P	S	14	2.6	0.2	
19680	11/15/01	103	4	M	1998	0.70766	0.92	W	TUO	661.5	100.4	91.8	110.4	S	S	S	.			
19684	11/15/01	92	3.5	M	1998	0.70776	0.95	W	TUO	578.3	86.1	77.5	96.2	S	S	S	.			
19685	11/15/01	87	4	F	1998	0.70811	0.89	W	TUO	545.7	80.6	72.0	90.6	S	P	S	.			
19687	11/15/01	82	3.5	M	1998	0.70806	0.95	W	TUO	557.0	82.5	73.9	92.5	S	P	S	16	3.5	0.3	
19691	11/15/01	91	4	M	1998	0.70721	0.19	W	TUO*	488.7	70.8	62.2	80.9	P	P	S	*Inconclusive natal assignment*			
19719	11/19/01	94.5	3.5	F	1998	0.70806	0.94	W	TUO	547.9	80.9	72.3	91.0	S	P	S	18	3.0	0.2	
19772	11/28/01	97	4	F	1998	0.70769	0.93	W	TUO	506.5	73.8	65.3	83.9	P	P	S	13	2.9	0.2	
19776	11/28/01	90	4	F	1998	0.70821	0.53	W	TUO	542.0	79.9	71.3	90.0	S	P	S	9	2.5	0.4	
19777	11/28/01	91	4	F	1998	0.70816	0.76	W	TUO	519.7	76.1	67.5	86.2	S	P	S	8	3.7	0.1	
19781	11/28/01	86	4	F	1998	0.70824	0.37	W	TUO*	532.5	78.3	69.7	88.4	S	P	S	*Inconclusive natal assignment*			
19783	11/28/01	89	4	F	1998	0.70793	0.98	W	TUO	480.4	69.4	60.8	79.5	P	P	S	16	3.0	0.2	
19785	11/28/01	88	4	F	1998	0.70765	0.91	W	TUO	540.6	79.7	71.1	89.8	S	P	S	34	3.2	0.3	
19790	11/28/01	94	4	F	1998	0.70818	0.68	W	TUO	583.5	87.0	78.4	97.1	S	S	S	32	2.9	0.2	
19796	12/03/01	81	4	F	1998	0.70811	0.88	W	TUO	566.3	84.1	75.5	94.1	S	S	S	17	3.8	0.1	
19798	12/03/01	93	4	M	1998	0.70814	0.83	W	TUO	525.6	77.1	68.5	87.2	S	P	S	17	4.0	0.2	
19800	12/03/01	114	4	M	1998	0.70776	0.95	W	TUO	595.1	89.0	80.4	99.1	S	S	S	.			
19802	12/03/01	97	4	F	1998	0.70824	0.40	W	TUO*	556.7	82.4	73.8	92.5	S	P	S	*Inconclusive natal assignment*			
19805	12/03/01	88	4	F	1998	0.70821	0.56	W	TUO	524.7	77.0	68.4	87.0	S	P	S	10	3.1	0.2	
19806	12/03/01	89	4	F	1998	0.70769	0.93	W	TUO	573.4	85.3	76.7	95.4	S	S	S	6	3.6	0.2	
19810	12/03/01	85	4	F	1998	0.70765	0.91	W	TUO	516.6	75.6	67.0	85.6	S	P	S	15	3.5	0.1	
19820	12/03/01	105	4	M	1998	0.70779	0.96	W	TUO	536.5	79.0	70.4	89.0	S	P	S	23	3.0	0.2	
19821	12/03/01	94	4	F	1998	0.70812	0.86	W	TUO	549.4	81.2	72.6	91.2	S	P	S	14	3.1	0.2	
19838	12/03/01	73	4	F	1998	0.70765	0.91	W	TUO	501.6	73.0	64.4	83.1	P	P	S	19	3.4	0.2	
19840	12/03/01	81	4	F	1998	0.70813	0.84	W	TUO	484.3	70.1	61.5	80.1	P	P	S	14	3.8	0.2	
19857	12/04/01	97	4	M	1998	0.70776	0.95	W	TUO	574.8	85.5	76.9	95.6	S	S	S	10	3.1	0.3	
19864	12/04/01	99	4	M	1998	0.70813	0.84	W	TUO	564.5	83.8	75.2	93.8	S	S	S	17	2.5	0.3	
19867	12/04/01	86	4	F	1998	0.70808	0.93	W	TUO	502.5	73.2	64.6	83.2	P	P	S	6	3.1	0.2	

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

19872	12/10/01	84	4	F	1998	0.70815	0.78	W	TUO	508.7	74.2	65.6	84.3	P	P	S	16	3.1	0.2	
19875	12/10/01	83	4	F	1998	0.70823	0.42	W	TUO*	526.6	77.3	68.7	87.4	S	P	S	*Inconclusive natal assignment*			
19879	12/10/01	76	4	F	1998	0.70766	0.91	W	TUO	530.5	78.0	69.4	88.0	S	P	S	15	2.5	0.1	
19880	12/10/01	89	4	F	1998	0.70765	0.91	W	TUO	506.0	73.8	65.2	83.8	P	P	S	32	3.3	0.3	
19881	12/10/01	99	4	F	1998	0.70813	0.85	W	TUO	586.0	87.4	78.9	97.5	S	S	S	23	2.8	0.4	
20183	11/28/01	101	4	F	1998	0.70773	0.94	W	TUO	577.9	86.1	77.5	96.1	S	S	S	11	4.5	0.2	
4492	11/28/00	57	2	M	1999	0.70804	0.95	W	TUO	603.8	90.5	81.9	100.6	S	S	S	n/a	n/a	n/a	Microstructure ran out before fish left natal river
4526	12/11/00	67	2	F	1999	0.70800	0.97	W	TUO	871.9	136.3	127.7	146.4	S	S	S	.			
11009	11/16/01	.	3	F	1999	0.70757	0.86	W	TUO	517.8	75.8	67.2	85.8	S	P	S	.			
11016	11/16/01	79.5	3	F	1999	0.70731	0.40	W	TUO*	465.7	66.9	58.3	76.9	P	P	S	24	3.1	0.2	
11019	11/16/01	60	3	F	1999	0.70743	0.67	INC	TUO*	548.1	81.0	72.4	91.0	S	P	S	.			Unreadable, so cannot assign natal location or do ageing
11021	11/16/01	73	3	F	1999	0.70731	0.39	W	TUO*	525.1	77.0	68.4	87.1	S	P	S	17	2.8	0.3	
11041	12/11/01	77	3	F	1999	0.70805	0.95	W	TUO	556.9	82.5	73.9	92.5	S	P	S	13	3.0	0.2	
11094	11/30/01	80	3	F	1999	0.70756	0.85	W	TUO	516.3	75.5	66.9	85.6	S	P	S	15	3.8	0.3	
11096	11/30/01	77	3	F	1999	0.70764	0.91	W	TUO	568.8	84.5	75.9	94.6	S	S	S	12	3.5	0.2	
11099	11/30/01	73	3	F	1999	0.70733	0.44	W	TUO*	491.3	71.3	62.7	81.3	P	P	S	*Inconclusive natal assignment*			
11100	11/30/01	83	3	F	1999	0.70748	0.75	W	TUO	572.6	85.2	76.6	95.2	S	S	S	.			
11132	11/26/01	76	3	F	1999	0.70734	0.46	W	TUO*	521.5	76.4	67.8	86.5	S	P	S	15	3.0	0.3	
11141	11/26/01	77	3	F	1999	0.70741	0.63	W	TUO	485.0	70.2	61.6	80.2	P	P	S	.			
11146	11/26/01	81	3	F	1999	0.70722	0.20	W	TUO*	487.0	70.5	61.9	80.6	P	P	S	*Inconclusive natal assignment*			
11157	11/26/01	80	3	M	1999	0.70740	0.61	W	TUO	467.5	67.2	58.6	77.3	P	P	S	8	5.6	0.1	
11161	11/26/01	74	3	F	1999	0.70792	0.98	W	TUO	519.2	76.0	67.4	86.1	S	P	S	.			
11162	11/26/01	78	3	F	1999	0.70724	0.25	W	TUO*	534.0	78.6	70.0	88.6	S	P	S	7	4.7	0.3	
11174	12/07/01	80	3	F	1999	0.70754	0.83	W	TUO	488.2	70.7	62.1	80.8	P	P	S	1	2.7	n/a	
11192	11/23/01	74	3	F	1999	0.70772	0.94	W	TUO	550.0	81.3	72.7	91.4	S	P	S	29	3.1	0.3	
11209	11/21/01	97	3	M	1999	0.70771	0.94	W	TUO	602.1	90.2	81.6	100.3	S	S	S	15	3.8	0.2	
11213	11/21/01	83	3	F	1999	0.70771	0.94	W	TUO	521.3	76.4	67.8	86.4	S	P	S	.			
11217	11/21/01	40.5	3	M	1999	0.70764	0.90	W	TUO	474.1	68.3	59.7	78.4	P	P	S	10	2.8	0.2	
14499	11/04/02	93	4	F	1999	0.70814	0.83	W	TUO	610.2	91.6	83.0	101.6	S	S	S	33	2.8	0.3	
14568	11/05/02	107	4	M	1999	0.70761	0.89	W	TUO	631.3	95.2	86.6	105.3	S	S	S	30	2.69	0.36	

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

14621	11/12/02	104	4	M	1999	0.70776	0.95	W	TUO	642.0	97.0	88.4	107.1	S	S	S	31	3.1	0.2	
14623	11/12/02	85	4	F	1999	0.70782	0.97	W	TUO	531.8	78.2	69.6	88.2	S	P	S	12	3.0	0.2	
14627	11/12/02	97	4	F	1999	0.70754	0.83	W	TUO	522.3	76.6	68.0	86.6	S	P	S	.			
14635	11/12/02	101	4	M	1999	0.70777	0.96	W	TUO	548.5	81.0	72.4	91.1	S	P	S	17	2.9	0.3	
14647	11/12/02	96	4	F	1999	0.70805	0.95	W	TUO	503.9	73.4	64.8	83.5	P	P	S	.			
14669	11/12/02	104	4	M	1999	0.70761	0.89	W	TUO	541.0	79.7	71.2	89.8	S	P	S	15	3.8	0.2	
14687	11/12/02	91	4	F	1999	0.70738	0.56	W	TUO	580.1	86.4	77.8	96.5	S	S	S	20	3.7	0.2	
14693	11/12/02	99	4	M	1999	0.70751	0.80	W	TUO	570.7	84.8	76.2	94.9	S	S	S	27	3.2	0.2	
14716	11/13/02	97	4	M	1999	0.70757	0.85	W	TUO	597.6	89.4	80.8	99.5	S	S	S	28	3.2	0.3	
14729	11/13/02	96	4	M	1999	0.70726	0.28	W	TUO*	533.1	78.4	69.8	88.5	S	P	S	20	3.7	0.3	
14759	11/14/02	101	4	M	1999	0.70766	0.91	W	TUO	563.5	83.6	75.0	93.7	S	S	S	22	2.3	0.2	
14774	11/14/02	86	4	F	1999	0.70786	0.97	W	TUO	528.7	77.6	69.1	87.7	S	P	S	.			
14804	11/14/02	93	4	F	1999	0.70768	0.93	W	TUO	543.8	80.2	71.6	90.3	S	P	S	18	3.9	0.2	
14824	11/15/02	98	4	M	1999	0.70733	0.44	W	TUO*	551.6	81.6	73.0	91.6	S	P	S	*Inconclusive natal assignment*			
14850	11/18/02	92	4	F	1999	0.70722	0.19	W	TUO*	513.4	75.0	66.4	85.1	S	P	S	*Inconclusive natal assignment*			
14884	11/18/02	89	4	F	1999	0.70749	0.77	W	TUO	545.5	80.5	71.9	90.6	S	P	S	.			
14889	11/18/02	88	4	M	1999	0.70754	0.82	W	TUO	576.1	85.8	77.2	95.8	S	S	S	22	3.7	0.2	
14892	11/18/02	100	4	M	1999	0.70725	0.26	W	TUO*	534.3	78.6	70.0	88.7	S	P	S	*Inconclusive natal assignment*			
14904	11/18/02	100	4	M	1999	0.70745	0.70	W	TUO	553.8	81.9	73.3	92.0	S	P	S	28	3.4	0.2	
14919	11/18/02	88	4	F	1999	0.70729	0.35	W	TUO*	458.5	65.6	57.1	75.7	P	P	S	*Inconclusive natal assignment*			
14953	11/19/02	103	4	M	1999	0.70787	0.97	W	TUO	534.2	78.6	70.0	88.6	S	P	S	16	2.7	0.3	
14955	11/19/02	94	4	F	1999	0.70813	0.84	W	TUO	562.1	83.4	74.8	93.4	S	P	S	8	3.4	0.3	
14976	11/19/02	102	4	M	1999	0.70765	0.91	W	TUO	524.7	77.0	68.4	87.0	S	P	S	5	3.9	0.1	
14999	11/20/02	104	4	M	1999	0.70726	0.27	W	TUO*	523.4	76.7	68.1	86.8	S	P	S	1	4.9		
15001	11/20/02	101	4	M	1999	0.70787	0.97	W	TUO	487.7	70.6	62.0	80.7	P	P	S	13	3.7	0.3	
15052	11/20/02	105	4	M	1999	0.70776	0.95	W	TUO	590.3	88.2	79.6	98.2	S	S	S	.			
15064	11/20/02	98	4	M	1999	0.70775	0.95	W	TUO	475.5	68.5	60.0	78.6	P	P	S	11	3.2	0.4	
15097	11/21/02	104	4	M	1999	0.70774	0.95	W	TUO	563.5	83.6	75.0	93.7	S	S	S	11	3.3	0.1	
15146	11/24/02	107	4	M	1999	0.70721	0.19	W	TUO*	513.8	75.1	66.5	85.2	S	P	S	*Inconclusive natal assignment*			
15150	11/24/02	108	4	M	1999	0.70761	0.89	W	TUO	505.1	73.6	65.0	83.7	P	P	S	22	3.6	0.3	
15165	11/24/02	100	4	M	1999	0.70726	0.27	W	TUO*	447.6	63.8	55.2	73.9	P	P	P	*Inconclusive natal assignment*			
19679	11/15/01	78	3	F	1999	0.70754	0.83	W	TUO	559.9	83.0	74.4	93.1	S	P	S	11	3.0	0.1	
19686	11/15/01	81	3	F	1999	0.70780	0.96	W	TUO	567.8	84.3	75.7	94.4	S	S	S	6	4.2	0.3	
19688	11/15/01	72.5	3	F	1999	0.70772	0.94	W	TUO	578.5	86.2	77.6	96.2	S	S	S	.			

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

19705	11/15/01	76	3	F	1999	0.70729	0.35	W	TUO*	440.8	62.6	54.0	72.7	P	F	P	*Inconclusive natal assignment*			
19722	11/19/01	87	3	F	1999	0.70733	0.44	W	TUO*	582.6	86.9	78.3	96.9	S	S	S	*Inconclusive natal assignment*			
19775	11/28/01	83	3	F	1999	0.70736	0.52	W	TUO	592.7	88.6	80.0	98.7	S	S	S	31	3.7	0.2	
19779	11/28/01	70	3	F	1999	0.70760	0.88	W	TUO	522.2	76.5	67.9	86.6	S	P	S	10	3.5	0.2	
19782	11/28/01	76	3	M	1999	0.70779	0.96	W	TUO	555.0	82.1	73.6	92.2	S	P	S	18	3.1	0.2	
19786	11/28/01	85	3	M	1999	0.70746	0.73	W	TUO	580.0	86.4	77.8	96.5	S	S	S	9	3.5	0.3	
19791	11/28/01	79	3	F	1999	0.70735	0.49	W	TUO*	508.6	74.2	65.6	84.3	P	P	S	*Inconclusive natal assignment*			
19792	11/28/01	76	3	F	1999	0.70814	0.83	W	TUO	520.8	76.3	67.7	86.4	S	P	S	5	4.1	0.1	
19797	12/03/01	84	3	F	1999	0.70735	0.48	W	TUO*	523.0	76.7	68.1	86.7	S	P	S	*Inconclusive natal assignment*			
19816	12/03/01	81	3	M	1999	0.70770	0.93	W	TUO	736.1	113.1	104.5	123.2	S	S	S	.			
19836	12/03/01	88	3	M	1999	0.70726	0.29	W	TUO*	486.4	70.4	61.8	80.5	P	P	S	*Inconclusive natal assignment*			
19841	12/03/01	74	3	F	1999	0.70724	0.23	W	TUO*	491.7	71.3	62.7	81.4	P	P	S	*Inconclusive natal assignment*			
19845	12/04/01	87	3	M	1999	0.70788	0.97	W	TUO	549.3	81.2	72.6	91.2	S	P	S	20	3.2	0.2	
19855	12/04/01	85	3	M	1999	0.70768	0.92	W	TUO	523.0	76.7	68.1	86.7	S	P	S	33	2.8	0.2	
19861	12/04/01	74	3	F	1999	0.70775	0.95	W	TUO	544.2	80.3	71.7	90.4	S	P	S	8	3.4	0.2	
19866	12/04/01	86	3	M	1999	0.70724	0.23	W	TUO*	487.4	70.6	62.0	80.6	P	P	S	9	3.1	0.2	
19868	12/04/01	74	3	F	1999	0.70736	0.51	W	TUO	645.4	97.6	89.0	107.7	S	S	S	9	3.7	0.2	
19874	12/10/01	75	3	F	1999	0.70756	0.84	W	TUO	409.6	57.3	48.7	67.4	P	F	P	8	2.73	0.1 4	
19876	12/10/01	71	3	M	1999	0.70765	0.91	W	TUO	538.8	79.4	70.8	89.4	S	P	S	16	3.2	0.2	
11055	11/20/01	56	2	M	2000	0.70770	0.93	W	TUO	433.6	61.4	52.8	71.5	P	F	P	.			
11063	11/20/01	58	2	M	2000	0.70763	0.90	W	TUO	614.4	92.3	83.7	102.4	S	S	S	53	2.8	0.2	
11076	11/20/01	81	2	F	2000	0.70807	0.93	W	TUO	557.2	82.5	73.9	92.6	S	P	S	5	3.1	0.1	
11083	11/20/01	59	2	M	2000	0.70742	0.65	W	TUO	529.8	77.8	69.2	87.9	S	P	S	35	3.2	0.2	
11111	11/08/01	54.5	2	F	2000	0.70775	0.95	W	TUO	563.7	83.6	75.0	93.7	S	S	S	24	4.0	0.3	
11133	11/26/01	59	2	F	2000	0.70797	0.97	W	TUO	472.4	68.0	59.4	78.1	P	P	S	.			
11167	11/26/01	60	2	F	2000	0.70752	0.81	W	TUO	512.3	74.8	66.3	84.9	P	P	S	33	3.4	0.3	
11212	11/21/01	65.5	2	F	2000	0.70760	0.88	W	TUO	559.7	82.9	74.4	93.0	S	P	S	32	3.8	0.2	
11215	11/21/01	60	2	F	2000	0.70795	0.98	W	TUO	577.4	86.0	77.4	96.0	S	S	S	20	4.3	0.3	
11220	10/31/01	62	2	F	2000	0.70789	0.97	W	TUO	601.9	90.2	81.6	100.2	S	S	S	30	2.7	0.3	
11223	10/31/01	54	2	M	2000	0.70784	0.97	W	TUO	571.0	84.9	76.3	94.9	S	S	S	33	3.4	0.3	
11228	10/31/01	60	2	M	2000	0.70778	0.96	W	TUO	519.3	76.0	67.4	86.1	S	P	S	30	3.7	0.3	
14528	11/04/02	95	3	M	2000	0.70813	0.85	W	TUO	558.3	82.7	74.1	92.8	S	P	S	24	3.3	0.2	
14539	11/04/02	72	3	F	2000	0.70789	0.97	W	TUO	519.7	76.1	67.5	86.2	S	P	S	28	3.2	0.1	

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14540	11/04/02	69	3	F	2000	0.70777	0.96	INC	TUO*	466.3	67.0	58.4	77.0	P	P	S		.		Unreadable, so cannot assign natal location or do ageing
14544	11/05/02	78	3	F	2000	0.70782	0.96	W	TUO	523.5	76.8	68.2	86.8	S	P	S	39	3.0	0.2	
14545	11/05/02	92	3	M	2000	0.70792	0.98	W	TUO	482.3	69.7	61.1	79.8	P	P	S	.			
14548	11/05/02	72	3	F	2000	0.70801	0.97	W	TUO	615.8	92.5	84.0	102.6	S	S	S	.			
14550	11/05/02	80	3	M	2000	0.70789	0.97	W	TUO	487.8	70.7	62.1	80.7	P	P	S	29	3.1	0.3	
14556	11/05/02	73	3	F	2000	0.70742	0.64	W	TUO	552.0	81.6	73.0	91.7	S	P	S	29	4.0	0.3	
14559	11/05/02	89	3	M	2000	0.70768	0.92	W	TUO	430.9	60.9	52.3	71.0	P	F	P	.			
14560	11/05/02	78	3	F	2000	0.70756	0.85	W	TUO	537.9	79.2	70.6	89.3	S	P	S	28	3.8	0.2	
14566	11/05/02	79	3	F	2000	0.70786	0.97	W	TUO	504.0	73.4	64.8	83.5	P	P	S	.			
14571	11/05/02	97	3	M	2000	0.70763	0.90	W	TUO	590.4	88.2	79.6	98.3	S	S	S	38	3.4	0.3	
14575	11/05/02	73	3	F	2000	0.70795	0.98	W	TUO	500.4	72.8	64.2	82.9	P	P	S	21	3.1	0.3	
14578	11/05/02	73	3	F	2000	0.70745	0.71	W	TUO	511.4	74.7	66.1	84.8	P	P	S	.			
14579	11/05/02	93	3	M	2000	0.70742	0.65	W	TUO	521.3	76.4	67.8	86.4	S	P	S	31	3.8	0.2	
14584	11/05/02	81	.	M	2000	0.70772	0.94	W	TUO	520.2	76.2	67.6	86.3	S	P	S	.			
14587	11/05/02	80	3	F	2000	0.70780	0.96	W	TUO	584.3	87.2	78.6	97.2	S	S	S	.			
14596	11/05/02	80	3	F	2000	0.70800	0.97	W	TUO	568.9	84.5	75.9	94.6	S	S	S	19	2.9	0.3	
14597	11/05/02	91	3	M	2000	0.70756	0.85	W	TUO	528.1	77.5	69.0	87.6	S	P	S	35	3.4	0.3	
14600	11/05/02	75	3	F	2000	0.70774	0.95	W	TUO	460.7	66.0	57.4	76.1	P	P	S	.			
14616	11/12/02	94	3	M	2000	0.70768	0.92	W	TUO	494.1	71.7	63.1	81.8	P	P	S	61	3.5	0.2	
14626	11/12/02	91	3	M	2000	0.70735	0.50	W	TUO	545.9	80.6	72.0	90.7	S	P	S	.			
14629	11/12/02	74	3	F	2000	0.70772	0.94	W	TUO	555.0	82.1	73.6	92.2	S	P	S	.			
14661	11/12/02	94	3	M	2000	0.70761	0.89	W	TUO	556.0	82.3	73.7	92.4	S	P	S	42	3.8	0.3	
14668	11/12/02	90	3	M	2000	0.70785	0.97	W	TUO	530.4	77.9	69.3	88.0	S	P	S	23	3.3	0.2	
14673	11/12/02	90	3	M	2000	0.70770	0.93	W	TUO	532.9	78.4	69.8	88.4	S	P	S	26	3.8	0.2	
14689	11/12/02	93	3	M	2000	0.70803	0.96	W	TUO	521.2	76.4	67.8	86.4	S	P	S	n/a	n/a	n/a	Microstructure ran out before fish left natal river
14701	11/13/02	93	3	M	2000	0.70803	0.96	W	TUO	456.0	65.2	56.6	75.3	P	P	S	0	n/a	n/a	Strange profile (used same distance for natal and FW exit)
14721	11/13/02	76	3	F	2000	0.70804	0.95	W	TUO	512.1	74.8	66.2	84.9	P	P	S	18	3.7	0.2	
14735	11/13/02	92	3	M	2000	0.70763	0.90	W	TUO	514.7	75.2	66.7	85.3	S	P	S	38	3.9	0.3	
14743	11/13/02	76	3	F	2000	0.70802	0.96	W	TUO	535.2	78.8	70.2	88.8	S	P	S	12	4.2	0.2	

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14749	11/14/02	80	3	F	2000	0.70773	0.94	W	TUO	473.3	68.2	59.6	78.2	P	P	S	34	2.6	0.2	
14753	11/14/02	84	3	M	2000	0.70774	0.95	W	TUO	525.1	77.0	68.4	87.1	S	P	S	31	4.1	0.3	
14769	11/14/02	81	3	F	2000	0.70757	0.85	W	TUO	503.2	73.3	64.7	83.4	P	P	S	31	3.7	0.3	
14783	11/14/02	89	3	M	2000	0.70793	0.98	W	TUO	564.4	83.8	75.2	93.8	S	S	S	12	4.6	0.2	
14785	11/14/02	95	3	M	2000	0.70766	0.91	W	TUO	535.2	78.8	70.2	88.8	S	P	S	.			
14786	11/14/02	76	3	F	2000	0.70781	0.96	W	TUO	505.4	73.7	65.1	83.7	P	P	S	23	4.1	0.3	
14813	11/14/02	86	3	M	2000	0.70799	0.97	W	TUO	500.7	72.9	64.3	82.9	P	P	S	25	3.4	0.3	
14815	11/15/02	82	3	F	2000	0.70761	0.89	W	TUO	501.9	73.1	64.5	83.1	P	P	S	.			
14858	11/18/02	104	3	M	2000	0.70740	0.61	W	TUO	484.4	70.1	61.5	80.1	P	P	S	3	2.0	0.0	
14880	11/18/02	80	3	M	2000	0.70727	0.31	W	TUO*	480.4	69.4	60.8	79.5	P	P	S	12	5.1	0.2	
14907	11/18/02	74	3	F	2000	0.70773	0.95	W	TUO	497.3	72.3	63.7	82.3	P	P	S	14	3.9	0.2	
14921	11/18/02	93	3.5	F	2000	0.70780	0.96	W	TUO	547.1	80.8	72.2	90.9	S	P	S	47	3.3	0.2	
14929	11/18/02	91	3	M	2000	0.70764	0.90	W	TUO	529.8	77.8	69.3	87.9	S	P	S	30	3.2	0.3	
14975	11/19/02	102	3	M	2000	0.70743	0.67	W	TUO	565.7	84.0	75.4	94.0	S	S	S	.			
15091	11/21/02	100	3	F	2000	0.70792	0.98	W	TUO	557.5	82.6	74.0	92.6	S	P	S	26 †	3.90	0.1 5	† Microstructure ran out 52um before FW exit (inferred 13 increments at end)
15113	11/21/02	100	3	M	2000	0.70763	0.90	W	TUO	495.1	71.9	63.3	82.0	P	P	S	.			
15133	11/24/02	103	3	M	2000	0.70754	0.83	W	TUO	513.7	75.1	66.5	85.1	S	P	S	23	3.8	0.2	
15193	11/24/02	101	3	M	2000	0.70773	0.94	W	TUO	611.3	91.8	83.2	101.8	S	S	S	31	3.1	0.3	
15243	12/02/02	91	3	M	2000	0.70777	0.95	W	TUO	524.0	76.8	68.3	86.9	S	P	S	22	3.5	0.2	
19681	11/15/01	65	2	M	2000	0.70759	0.87	W	TUO	478.4	69.1	60.5	79.1	P	P	S	.			
19695	11/19/01	59.5	2	M	2000	0.70759	0.88	W	TUO	540.6	79.7	71.1	89.7	S	P	S	25	3.2	0.3	
19813	12/03/01	48	2	M	2000	0.70789	0.97	W	TUO	561.0	83.2	74.6	93.2	S	P	S	48	3.4	0.3	
19831	12/03/01	57	2	F	2000	0.70779	0.96	W	TUO	498.5	72.5	63.9	82.5	P	P	S	28	4.8	0.3	
19853	12/04/01	60	2	F	2000	0.70781	0.96	W	TUO	544.3	80.3	71.7	90.4	S	P	S	7	4.0	0.2	
19858	12/04/01	58	2	F	2000	0.70761	0.89	W	TUO	487.6	70.6	62.0	80.7	P	P	S	.			
17628	11/14/05	91	3	M	2003	0.70730	0.37	W	TUO*	551.7	81.6	73.0	91.6	S	P	S	19	3.2	0.3	
17631	11/14/05	84	3	F	2003	0.70751	0.80	W	TUO	594.7	88.9	80.4	99.0	S	S	S	12	4.6	0.2	
17634	11/14/05	81	3	F	2003	0.70772	0.94	W	TUO	511.9	74.8	66.2	84.8	P	P	S	9	2.9	0.1	
17637	11/16/05	92	3	M	2003	0.70726	0.27	W	TUO*	457.4	65.5	56.9	75.5	P	P	S	*Inconclusive natal assignment*			
17638	11/16/05	76	3	F	2003	0.70731	0.41	W	TUO*	558.9	82.8	74.2	92.9	S	P	S	11	3.1	0.2	
17645	11/21/05	75	3	F	2003	0.70747	0.74	W	TUO	628.3	94.7	86.1	104.7	S	S	S	.			

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive (“INC”), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

17651	11/21/05	88	3	M	2003	0.70745	0.71	W	TUO	582.0	86.8	78.2	96.8	S	S	S	8	4.4	0.2	
17654	11/21/05	73	3	F	2003	0.70757	0.86	W	TUO	380.8	52.4	43.8	62.4	F	F	P	16	3.6	0.1	
17666	11/28/05	73	3	F	2003	0.70753	0.82	W	TUO	529.0	77.7	69.1	87.8	S	P	S	5	2.9	0.3	
17667	11/28/05	75	3	F	2003	0.70744	0.69	W	TUO	531.9	78.2	69.6	88.3	S	P	S	18	2.9	0.3	
17669	11/28/05	72	3	F	2003	0.70743	0.66	W	TUO	597.8	89.5	80.9	99.5	S	S	S	.			
17672	11/28/05	79	3	F	2003	0.70756	0.85	INC	TUO*		n/a (vaterite)						.			Otolith vateritic during natal rearing (so no HvW assignment or exit age/dist)
17673	11/28/05	71	3	F	2003	0.70745	0.70	W	TUO		73.8	65.2	83.8	P	P	S	.			
17679	11/28/05	85	3	M	2003	0.70729	0.34	W	TUO*		82.3	73.7	92.4	S	P	S	21	4.6	0.3	
17680	11/28/05	75	3	F	2003	0.70777	0.96	W	TUO		86.6	78.0	96.6	S	S	S	n/a	n/a	n/a	Microstructure ran out before fish left natal river
17681	11/28/05	72	3	F	2003	0.70763	0.90	W	TUO		67.5	58.9	77.6	P	P	S	.			
17685	11/28/05	61	3	M	2003	0.70727	0.30	W	TUO*		74.7	66.1	84.8	P	P	S	*Inconclusive natal assignment*			
17690	11/28/05	83	3	M	2003	0.70754	0.83	W	TUO		91.5	82.9	101.5	S	S	S	8	3.1	0.3	
17692	11/29/05	85	3	F	2003	0.70759	0.87	W	TUO		73.9	65.4	84.0	P	P	S	2	4.3	0.1	
17703	12/06/05	75	3	F	2003	0.70751	0.79	W	TUO		96.9	88.3	106.9	S	S	S	14	3.1	0.2	
17712	12/06/05	90	3	M	2003	0.70734	0.46	W	TUO*		79.8	71.2	89.8	S	P	S	*Inconclusive natal assignment*			
17713	12/06/05	76	3	F	2003	0.70755	0.84	W	TUO		70.3	61.7	80.3	P	P	S	5	4.4	0.2	
17716	12/06/05	82	3	F	2003	0.70744	0.69	W	TUO		85.0	76.4	95.1	S	S	S	.			
17718	12/07/05	79	3	F	2003	0.70751	0.79	W	TUO		86.7	78.1	96.8	S	S	S	.			
17729	12/12/05	92	3	M	2003	0.70754	0.82	W	TUO		81.7	73.1	91.8	S	P	S	.			
17740	12/12/05	79	3	F	2003	0.70738	0.56	W	TUO		73.6	65.0	83.6	P	P	S	.			
17742	12/12/05	75	3	F	2003	0.70743	0.66	W	TUO		74.6	66.0	84.6	P	P	S	19	3.8	0.2	
17746	12/12/05	72	3	F	2003	0.70762	0.89	W	TUO		94.7	86.1	104.8	S	S	S	.			
17751	12/12/05	85	3	F	2003	0.70755	0.84	W	TUO		83.2	74.6	93.3	S	P	S	9	3.7	0.1	
17753	12/12/05	81	3	M	2003	0.70750	0.79	W	TUO		73.2	64.7	83.3	P	P	S	5	4.1	0.1	
17758	12/12/05	84	3	F	2003	0.70718	0.14	W	TUO*		78.1	69.5	88.2	S	P	S	8	3.6	0.3	
17759	12/12/05	70	3	F	2003	0.70765	0.91	W	TUO		78.3	69.8	88.4	S	P	S	.			
17763	12/12/05	79	3	F	2003	0.70744	0.70	W	TUO		75.9	67.3	86.0	S	P	S	13	3.9	0.2	
18144	12/05/06	86	4	F	2003	0.70726	0.29	W	TUO*		80.7	72.1	90.8	S	P	S	16	3.1	0.1	
18150	12/11/06	84	4	F	2003	0.70741	0.62	W	TUO		72.3	63.7	82.4	P	P	S	15	3.2	0.2	
24120	11/07/11	76	3	F	2009	0.70777	0.73	W	TUO		73.9	65.4	84.0	P	P	S	24	3.1	0.3	

Appendix 1B Table showing capture details of adult samples, their natal assignment and reconstructed fork length (FL) and age at exit from the freshwater delta (FW exit). The probability of the assignment to the Tuolumne (TUO) based on Sr isotopes is indicated, as well as the results of the otolith microstructure analyses to separate hatchery from wild fish (H vs. W). Where otoliths were unreadable, and microstructure analyses inconclusive ("INC"), or when the probability of assignment to TUO was <0.5 based on mean Sr isotopic values, the natal location is marked by an asterisk. Increment information is marked with (.) if otolith was unreadable.The life stage at FW exit was categorized as fry (F: FL<55mm), parr (P: FL >55mm to <75mm) or smolt (S: FL >75mm).

24176	11/14/11	81	3	F	2009	0.70781	0.77	W	TUO		91.3	82.7	101.4	S	S	S	20	2.9	0.4	
24178	11/14/11	67	3	M	2009	0.70778	0.74	W	TUO		88.0	79.4	98.1	S	S	S	13	3.5	0.3	
24238	11/21/11	70	3	F	2009	0.70773	0.69	W	TUO		80.1	71.5	90.2	S	P	S	.			
24283	11/23/11	83	3	M	2009	0.70734	0.16	W	TUO*		80.1	71.5	90.2	S	P	S	*Inconclusive natal assignment*			
24292	11/28/11	95	3	M	2009	0.70730	0.12	W	TUO*		91.4	82.8	101.4	S	S	S	*Inconclusive natal assignment*			
26012	11/13/12	84	4	F	2009	0.70780	0.76	W	TUO		83.7	75.1	93.8	S	S	S	7	2.6	0.1	

[1] Assignments using isotope-based discriminant function analysis and reference samples from existing or ongoing projects ([1], [2], P. Weber, A. Sturrock, unpub)
[2] Hatchery vs. wild assignment using microstructure-based discriminant function analysis and existing reference samples, after [3].
[3] Size-defined life stage designations (fry: <55mm, parr: >55mm to <75mm, smolt: >75mm), after [4].

1. Barnett-Johnson, R., et al., *Tracking natal origins of salmon using isotopes, otoliths, and landscape geology.* Limnology and Oceanography, 2008. **53**(4): p. 1633-1642.
2. Ingram, L.B. and P.K. Weber, *Salmon origin in California's Sacramento–San Joaquin river system as determined by otolith strontium isotopic composition.* Geology, 1999. **27**(9): p. 851-854.
3. Barnett-Johnson, R., et al., *Identifying the contribution of wild and hatchery Chinook salmon (Oncorhynchus tshawytscha) to the ocean fishery using otolith microstructure as natural tags.* Canadian Journal of Fisheries and Aquatic Sciences, 2007. **64**(12): p. 1683-1692.
4. Miller, J.A., A. Gray, and J. Merz, *Quantifying the contribution of juvenile migratory phenotypes in a population of Chinook salmon Oncorhynchus tshawytscha.* Marine Ecology Progress Series, 2010. **408**: p. 227-240.

Appendix 2 Capture details and natal assignments of strays to the Tuolumne River from outmigration years 1998, 1999, 2000, 2003 and 2009. The natal assignments were primarily based on otolith Sr isotopes, however where there was ambiguity in the assignment, otolith microstructure analyses were used to separate hatchery from wild fish (HvW). Site codes are provided in Table 2 of the main report.

ASN	Outmigration year	Date	Age	Length	Sex	Natal location	HvW
4184	1998	10/17/2000	3	84	F	X	H
4188	1998	10/19/2000	3	79.5	F	MOH	H
4190	1998	10/24/2000	3	91	M	MOH	H
4224	1998	10/25/2000	3	91	M	X	H
4227	1998	10/25/2000	3	87	M	X	H
4235	1998	10/25/2000	3	91	M	FEH	H
4236	1998	10/25/2000	3	72	F	MEH	n/a
4250	1998	10/30/2000	3	80	F	MOH	H
4260	1998	10/30/2000	3	78.5	M	X	H
4268	1998	10/30/2000	3	77	F	MOH	H
4273	1998	10/31/2000	3	78	F	MOK	W
4282	1998	10/31/2000	3	87	M	MEH	n/a
4285	1998	10/31/2000	3	77.5	F	MEH	n/a
4286	1998	10/31/2000	3	80	F	MEH	n/a
4289	1998	10/31/2000	3	83	F	MEH	n/a
4302	1998	11/6/2000	3	81.5	F	X	H
4313	1998	11/6/2000	3	92	F	MEH	H
4314	1998	11/6/2000	3	76	F	MEH	n/a
4324	1998	11/6/2000	3	77	F	MEH	H
4336	1998	11/7/2000	3	87	M	MEH	n/a
4338	1998	11/7/2000	3	84	F	MEH	n/a
4344	1998	11/7/2000	3	68	M	MEH	n/a
4349	1998	11/7/2000	3	75	F	MEH	n/a
4382	1998	11/13/2000	3	87	F	MEH	n/a
4396	1998	11/13/2000	3	92.5	M	MEH	n/a
4402	1998	11/14/2000	3	75	F	X	H
4406	1998	11/15/2000	3	88	M	MEH	n/a
4416	1998	11/15/2000	3	75	F	MEH	n/a
4422	1998	11/14/2000	3	80	F	MEH	n/a
4453	1998	11/20/2000	3	97	F	STA	W
4457	1998	11/20/2000	3	75	F	MEH	n/a
4467	1998	11/21/2000	3	92	F	STA	n/a
4479	1998	11/27/2000	3	63.5	F	MEH	n/a
4491	1998	11/28/2000	3	54	F	MEH	H
4495	1998	11/28/2000	3	86	F	X	H
4498	1998	11/29/2000	3	82	F	X	H
4503	1998	12/4/2000	3	83	F	X	H
4529	1998	12/12/2000	3	67	F	X	H
4530	1998	12/12/2000	3	61	F	X	H
9534	1998	7/7/2000	3	68	F	MOH	H
9551	1998	8/11/2000	3	74	F	MOH	H
11067	1998	11/20/2001	4	88	F	MEH	n/a
11095	1998	11/29/2001	4	86	F	MEH	n/a
11145	1998	11/26/2001	4	95	F	MEH	n/a
11147	1998	11/26/2001	4	93	F	MEH	n/a
11149	1998	11/26/2001	4	118	M	MEH	H
11150	1998	11/26/2001	4	84	M	MEH	n/a
11153	1998	11/26/2001	4	110	M	MEH	H
11156	1998	11/26/2001	4	92	F	MEH	n/a
11165	1998	11/26/2001	4	87	F	X	H
11170	1998	11/26/2001	4	95	F	MOH	H
11171	1998	11/26/2001	4	84	F	X	H
11172	1998	11/26/2001	4	83	F	MEH	H
11175	1998	12/7/2001	4	96	M	X	H
11178	1998	12/7/2001	4	88	F	MEH	H

Appendix 2 Capture details and natal assignments of strays to the Tuolumne River from outmigration years 1998, 1999, 2000, 2003 and 2009. The natal assignments were primarily based on otolith Sr isotopes, however where there was ambiguity in the assignment, otolith microstructure analyses were used to separate hatchery from wild fish (HvW). Site codes are provided in Table 2 of the main report.

11180	1998	12/19/2001	4	90	F	MER	W
11208	1998	11/21/2001	4	84	F	MOH	H
19676	1998	11/15/2001	4	103	M	STA	W
19766	1998	11/27/2001	3.5	84	F	MEH	n/a
19804	1998	12/3/2001	4	98	M	MEH	n/a
19814	1998	12/3/2001	4	91	F	MOK	W
19825	1998	12/3/2001	4	96	M	MEH	n/a
19839	1998	12/3/2001	4	82	F	MEH	n/a
19843	1998	12/3/2001	4	87	F	X	H
19848	1998	12/4/2001	3.5	85	F	STA	W
19856	1998	12/4/2001	4	103	M	STA	W
4375	1999	11/8/2000	2	57.5	F	THE	n/a
4404	1999	11/15/2000	2	56	M	MEH	n/a
4405	1999	11/15/2000	2	57	M	MEH	n/a
4468	1999	11/21/2000	2	37	F	MOH	H
4536	1999	12/20/2000	2	52	M	NIH	n/a
9548	1999	7/28/2000	2	81	F	MOH	H
9549	1999	8/4/2000	2	78	F	MOH	H
11011	1999	11/16/2001	3	77	F	FEH	H
11075	1999	11/20/2001	3	92.5	M	MOH	H
11077	1999	11/20/2001	3	91	F	MEH	H
11091	1999	11/20/2001	3	81	F	MOH	H
11148	1999	11/26/2001	3	72	F	MOH	H
11159	1999	11/26/2001	3	77	F	MOH	H
11168	1999	11/26/2001	3	71	F	THE	n/a
11169	1999	11/26/2001	3	75	F	MOH	H
11179	1999	12/7/2001	3	93	M	NIH	n/a
11183	1999	12/17/2001	3	80	M	NIH	n/a
14525	1999	11/4/2002	4	99	M	MOH	H
14546	1999	11/5/2002	4	95	M	MOH	H
14639	1999	11/12/2002	4	99	M	FEH	H
14640	1999	11/12/2002	4	88	F	STA	n/a
14641	1999	11/12/2002	4	96	F	FEH	H
14644	1999	11/12/2002	4	103	M	MOH	H
14645	1999	11/12/2002	4	101	M	MEH	n/a
14651	1999	11/12/2002	4	101	M	STA	n/a
14692	1999	11/12/2002	4	90	F	MOH	H
14711	1999	11/13/2002	4	94	M	MOH	H
14736	1999	11/13/2002	4	95	M	X	H
14737	1999	11/13/2002	4	110	M	X	H
14800	1999	11/14/2002	4	104	M	MOH	H
14827	1999	11/16/2002	4	98	F	STA	n/a
14828	1999	11/16/2002	4	99	M	X	H
14839	1999	11/18/2002	4	103	M	X	H
14877	1999	11/18/2002	4	90	M	STA	n/a
14883	1999	11/18/2002	4	90	M	STA	n/a
14906	1999	11/18/2002	4	100	M	MOH	H
14908	1999	11/18/2002	4	101	M	MOH	H
14912	1999	11/18/2002	4	102	M	MOH	H
14931	1999	11/18/2002	4	92	F	X	H
14944	1999	11/19/2002	4	101	M	MOH	H
14997	1999	11/20/2002	4	103	M	STA	n/a
15015	1999	11/20/2002	4	103	M	MOH	H
15098	1999	11/21/2002	4	96	M	MOH	H
15112	1999	11/21/2002	4	95	F	NIH	n/a
15114	1999	11/21/2002	4	100	M	MOH	H

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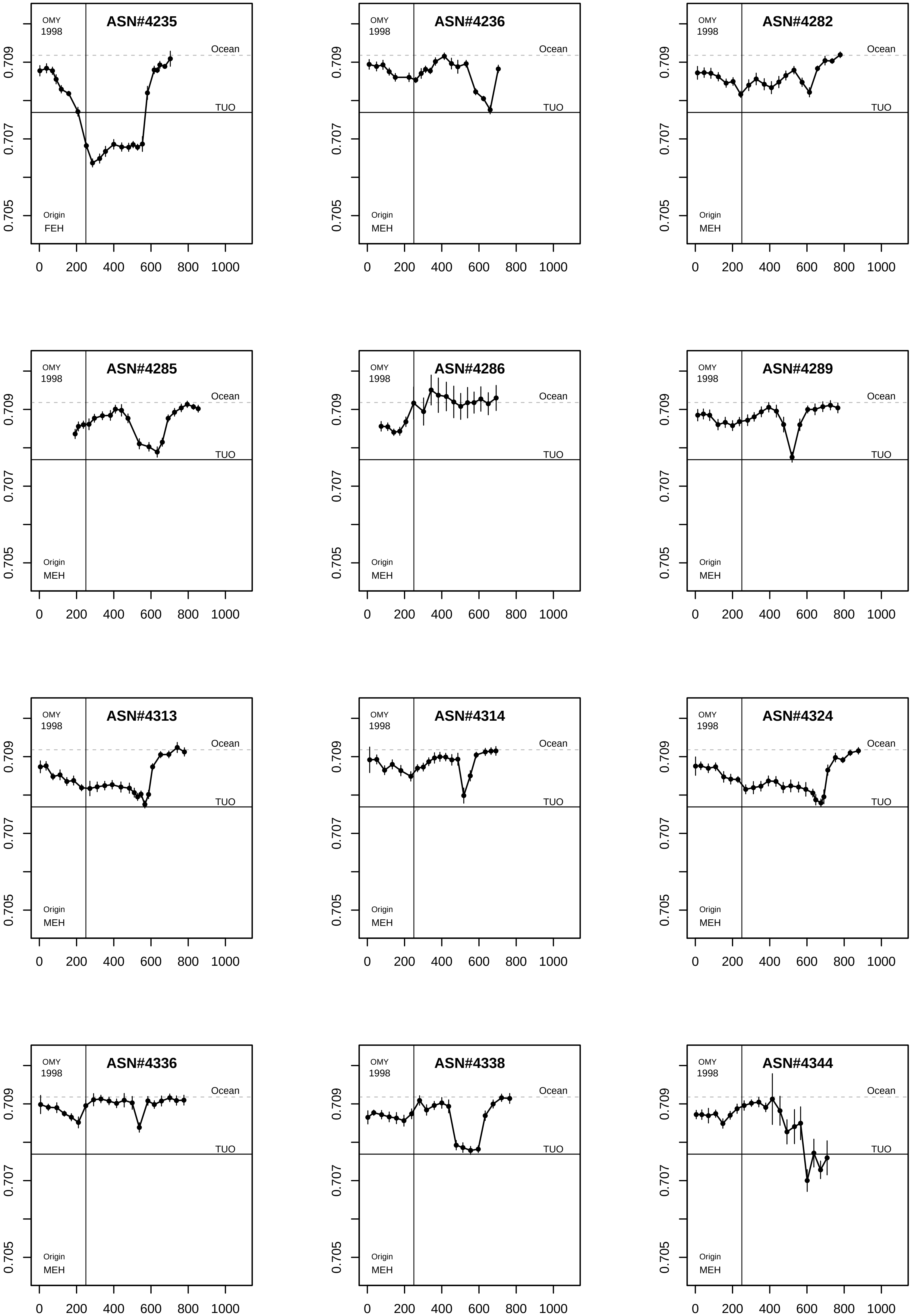
15124	1999	11/22/2002	4	95	F	STA	n/a
15127	1999	11/22/2002	4	102	M	STA	n/a
15131	1999	11/24/2002	4	100	M	MOK	n/a
15172	1999	11/24/2002	4	101	M	FEH	H
15178	1999	11/24/2002	4	104	F	MEH	H
15191	1999	11/24/2002	4	100	M	MOH	H
15216	1999	11/25/2002	4	98	M	FEH	H
15231	1999	11/26/2002	3.5	86	F	MOH	H
15236	1999	11/27/2002	4	100	M	NIH	n/a
15262	1999	12/3/2002	4	108	M	X	H
15269	1999	12/4/2002	4	102	M	X	H
15273	1999	12/5/2002	4	72	F	MOH	H
19678	1999	11/15/2001	3	82	F	MOH	H
19682	1999	11/15/2001	3	80	F	MEH	n/a
19689	1999	11/15/2001	3	88	F	X	H
19700	1999	11/15/2001	3	78	F	MEH	n/a
19778	1999	11/28/2001	3	77	F	MOH	H
19784	1999	11/28/2001	3	70	F	X	H
19787	1999	11/28/2001	3	89	F	X	H
19807	1999	12/3/2001	3	.		MEH	H
19832	1999	12/3/2001	3	69	F	MOH	H
19865	1999	12/4/2001	3	70	F	MOH	H
19870	1999	12/4/2001	3	75	M	X	H
19873	1999	12/10/2001	3	80	F	MEH	H
11012	2000	11/16/2001	2	58	F	X	H
11025	2000	11/9/2001	2	66	M	X	H
11062	2000	11/20/2001	2	86	M	FEH	H
11078	2000	11/20/2001	2	59	M	MEH	n/a
11079	2000	11/20/2001	2	55	M	MEH	n/a
11080	2000	11/20/2001	2	63	F	MOH	H
11103	2000	11/8/2001	2	57	M	MEH	n/a
11144	2000	11/26/2001	2	61	F	MEH	n/a
11184	2000	12/18/2001	2	54	M	NIH	n/a
11198	2000	11/21/2001	2	55.5	F	MOH	H
14486	2000	11/4/2002	3	110	M	MOH	H
14522	2000	11/4/2002	3	83	F	MOH	H
14524	2000	11/4/2002	3	77	F	MOH	H
14529	2000	11/4/2002	3	75	F	MOH	H
14547	2000	11/5/2002	3	79	M	MOH	H
14551	2000	11/5/2002	3	77	F	MOH	H
14569	2000	11/5/2002	3	81	M	MOH	H
14572	2000	11/5/2002	3	94	M	MOH	H
14577	2000	11/5/2002	3	99	M	FEH	H
14607	2000	11/6/2002	3	92	M	MOH	H
14612	2000	11/7/2002	3	98	M	MOH	H
14646	2000	11/12/2002	3	92	M	MOH	H
14657	2000	11/12/2002	3	76	F	MOH	H
14660	2000	11/12/2002	3	104	M	STA	W
14672	2000	11/12/2002	3	93	M	X	H
14744	2000	11/14/2002	3	84	M	X	H
14746	2000	11/14/2002	3	75	F	STA	W
14758	2000	11/14/2002	3	85	M	FEH	H
14763	2000	11/14/2002	3	79	F	X	H
14766	2000	11/14/2002	3	85	M	MOH	H
14890	2000	11/18/2002	3	87	M	MOH	H
14893	2000	11/18/2002	3	72	F	MOH	H

Appendix 2 Capture details and natal assignments of strays to the Tuolumne River from outmigration years 1998, 1999, 2000, 2003 and 2009. The natal assignments were primarily based on otolith Sr isotopes, however where there was ambiguity in the assignment, otolith microstructure analyses were used to separate hatchery from wild fish (HvW). Site codes are provided in Table 2 of the main report.

14895	2000	11/18/2002	3	75	F	MOH	H
14900	2000	11/18/2002	3	76	F	MOH	H
15025	2000	11/20/2002	3	100	M	STA	W
15067	2000	11/20/2002	3	99	M	MOH	H
15105	2000	11/21/2002	3	100	M	MOH	H
15128	2000	11/24/2002	3	107	M	STA	n/a
15159	2000	11/24/2002	3	105	M	FEH	H
19768	2000	11/27/2001	2	60	F	MOH	H
19789	2000	11/28/2001	2	64	F	MOH	H
19882	2000	12/10/2001	2	58	F	MEH	n/a
17621	2003	11/14/2005	3	75	F	MEH	n/a
17623	2003	11/14/2005	3	73	F	THE	n/a
17630	2003	11/14/2005	3	67	F	MOH	H
17632	2003	11/14/2005	3	65	F	THE	n/a
17641	2003	11/21/2005	3	70	F	MEH	n/a
17644	2003	11/21/2005	3	84	M	MOH	H
17647	2003	11/21/2005	3	85	F	MOH	H
17653	2003	11/21/2005	3	75	F	MOH	H
17658	2003	11/21/2005	3	74	F	MOH	H
17659	2003	11/21/2005	3	73	F	MOH	H
17661	2003	11/21/2005	3	75	F	MOH	H
17663	2003	11/21/2005	3	90	M	MEH	n/a
17674	2003	11/28/2005	3	74	F	NIH	n/a
17675	2003	11/28/2005	3	65	F	MOH	H
17676	2003	11/28/2005	3	79	F	MOH	H
17677	2003	11/28/2005	3	75	F	MOH	H
17686	2003	11/28/2005	3	70	M	NIH	n/a
17687	2003	11/28/2005	3	65	F	MOH	H
17688	2003	11/28/2005	3	76	F	MOH	H
17689	2003	11/28/2005	3	76	F	MEH	n/a
17694	2003	12/6/2005	3	77	F	MOH	H
17696	2003	12/6/2005	3	78	M	MOH	H
17697	2003	12/6/2005	3	81	F	NIH	n/a
17698	2003	12/6/2005	3	80	F	NIH	n/a
17704	2003	12/6/2005	3	81	F	MOH	H
17705	2003	12/6/2005	3	85	M	NIH	n/a
17707	2003	12/6/2005	3	84	M	MOH	H
17708	2003	12/6/2005	3	69	F	MOH	H
17709	2003	12/6/2005	3	79	F	NIH	n/a
17710	2003	12/6/2005	3	78	F	MOH	H
17719	2003	12/7/2005	3	80	M	MOH	H
17720	2003	12/7/2005	3	90	M	MOH	H
17721	2003	12/7/2005	3	79	F	NIH	n/a
17724	2003	12/12/2005	3	74	F	FEH	H
17726	2003	12/12/2005	3	80	F	NIH	n/a
17727	2003	12/12/2005	3	76	F	MOH	H
17730	2003	12/12/2005	3	79	M	MOH	H
17731	2003	12/12/2005	3	78	F	MOH	H
17732	2003	12/12/2005	3	69	F	MOH	H
17737	2003	12/12/2005	3	82	F	MOH	H
17739	2003	12/12/2005	3	77	F	THE	n/a
17741	2003	12/12/2005	3	77	F	FEH	H
17743	2003	12/12/2005	3	92	M	MOH	H
17744	2003	12/12/2005	3	75	F	FEH	H
17745	2003	12/12/2005	3	70	F	FEH	H
17747	2003	12/12/2005	3	78	F	MOH	H

Appendix 2 Capture details and natal assignments of strays to the Tuolumne River from outmigration years 1998, 1999, 2000, 2003 and 2009. The natal assignments were primarily based on otolith Sr isotopes, however where there was ambiguity in the assignment, otolith microstructure analyses were used to separate hatchery from wild fish (HvW). Site codes are provided in Table 2 of the main report.

17748	2003	12/12/2005	3	71	F	MOH	H
17749	2003	12/12/2005	3	78	F	MOH	H
17752	2003	12/12/2005	3	82	M	MOH	H
17754	2003	12/12/2005	3	73	F	FEH	H
17755	2003	12/12/2005	3	79	F	NIH	H
17760	2003	12/12/2005	3	74	F	MOH	H
17761	2003	12/12/2005	3	73	F	MOH	H
17762	2003	12/12/2005	3	83	F	MOH	H
18082	2003	11/14/2006	4	92	F	MOH	H
18095	2003	11/20/2006	4	78	F	MOH	H
18096	2003	11/20/2006	4	76	F	CNH	n/a
18101	2003	11/20/2006	4	88	F	MOH	H
18121	2003	11/21/2006	4	95	M	FEH	H
18129	2003	11/27/2006	4	88	F	MOH	H
18142	2003	12/4/2006	4	86	F	MOH	H
20197	2009	11/1/2010	2	63	M	CNH	n/a
20199	2009	11/1/2010	2	74	M	THE	H
20203	2009	11/1/2010	2	67	M	CNH	n/a
20204	2009	11/1/2010	2	63	M	CNH	n/a
20207	2009	11/8/2010	2	50	M	CNH	n/a
20218	2009	11/15/2010	2	59	M	MEH	n/a
20231	2009	11/15/2010	2	61	M	CNH	n/a
20239	2009	11/15/2010	2	60	M	CNH	n/a
20241	2009	11/15/2010	2	60	M	CNH	n/a
20242	2009	11/15/2010	2	62	M	CNH	n/a
20248	2009	11/17/2010	2	65	M	CNH	n/a
20249	2009	11/17/2010	2	68	M	CNH	n/a
20256	2009	11/22/2010	2	68	M	CNH	n/a
20264	2009	11/23/2010	2	63	M	MEH	n/a
24015	2009	10/3/2011	3	78	F	THE	n/a
24035	2009	10/10/2011	3	81	M	FEH	H
24038	2009	10/17/2011	3	77	F	FEA	W
24043	2009	10/17/2011	3	83	M	FEH	H
24052	2009	10/24/2011	3	81	F	FEH	H
24054	2009	10/24/2011	3	83	F	CNH	n/a
24056	2009	10/24/2011	3	82	F	CNH	n/a
24059	2009	10/24/2011	3	86	M	CNH	n/a
24065	2009	10/24/2011	3	82	F	CNH	n/a
24066	2009	10/24/2011	3	92	M	CNH	n/a
24112	2009	11/7/2011	3	77	M	FEH	H
24114	2009	11/7/2011	3	78	F	CNH	n/a
24117	2009	11/7/2011	3	86	M	CNH	n/a
24131	2009	11/7/2011	3	75	F	CNH	n/a
24141	2009	11/7/2011	3	77	F	CNH	n/a
24164	2009	11/9/2011	3	82	F	CNH	n/a
24168	2009	11/9/2011	3	87	M	CNH	n/a
24174	2009	11/14/2011	3	72	F	CNH	H
24177	2009	11/14/2011	3	78	F	CNH	n/a
24193	2009	11/14/2011	3	88	M	CNH	n/a
24214	2009	11/14/2011	3	85	F	CNH	n/a
24239	2009	11/21/2011	3	81	F	MOH	H
24290	2009	11/28/2011	3	75	F	NIH	n/a
24410	2009	12/12/2011	3	73	F	NIH	n/a
25886	2009	11/6/2012	4	81	F	THE	n/a



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Ocean
TUO
Origin
FEH
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0.707
0.705
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OMY
1998
ASN#4236
Ocean
TUO
Origin
MEH
OMY
1998
ASN#4282
Ocean
TUO
Origin
MEH
OMY
1998
ASN#4285
Ocean
TUO
Origin
MEH
OMY
1998
ASN#4286
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TUO
Origin
MEH
OMY
1998
ASN#4289
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TUO
Origin
MEH
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TUO
Origin
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1998
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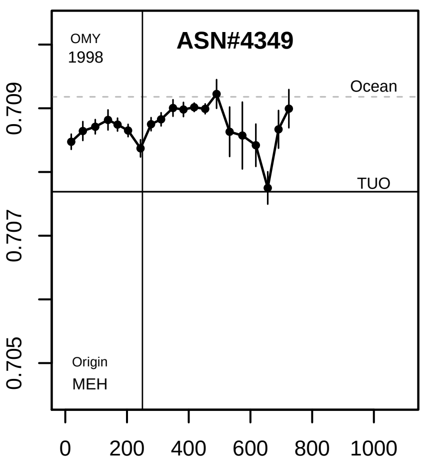
TUO
Origin
MEH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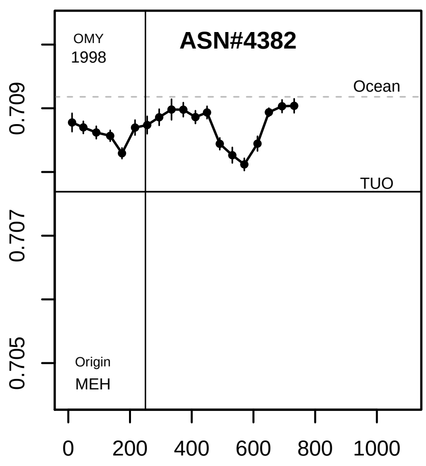
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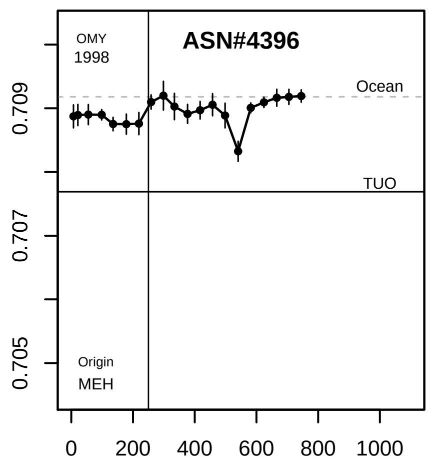
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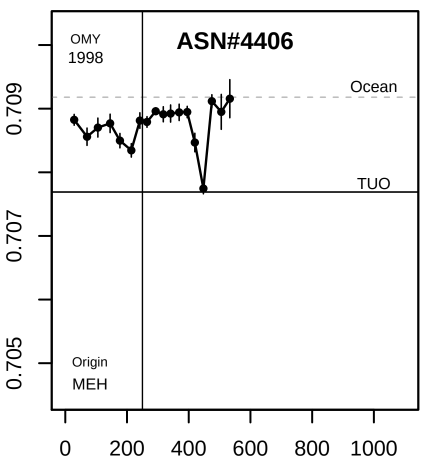
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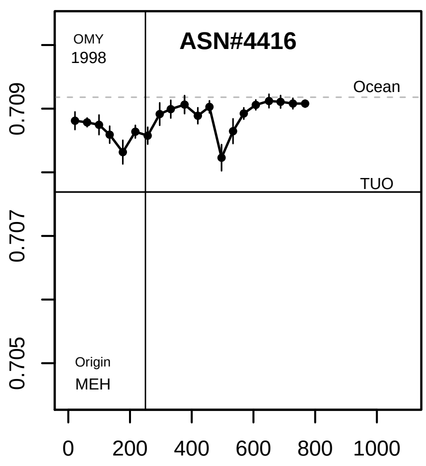
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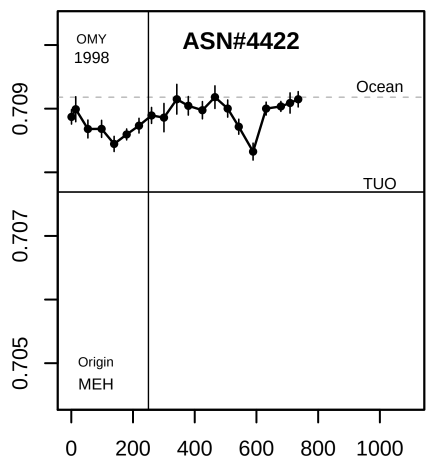
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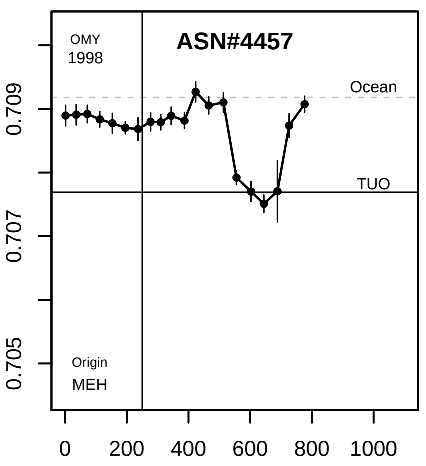
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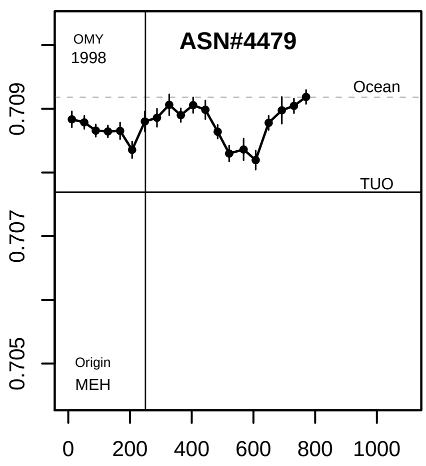
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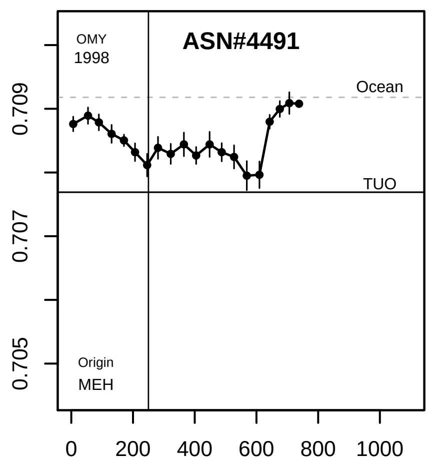
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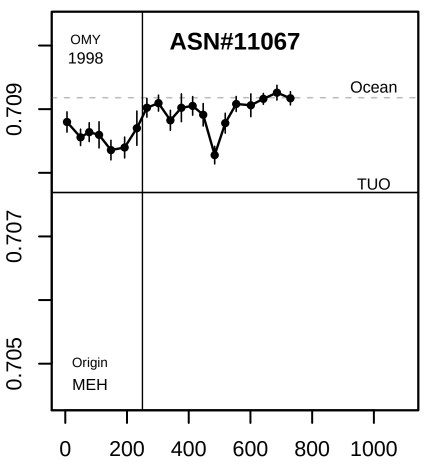
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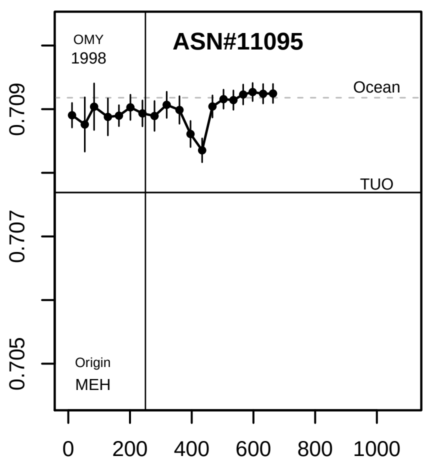
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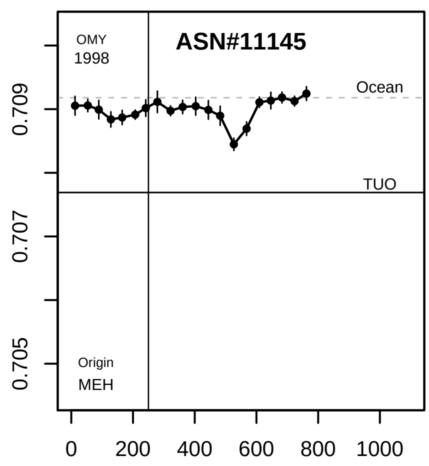
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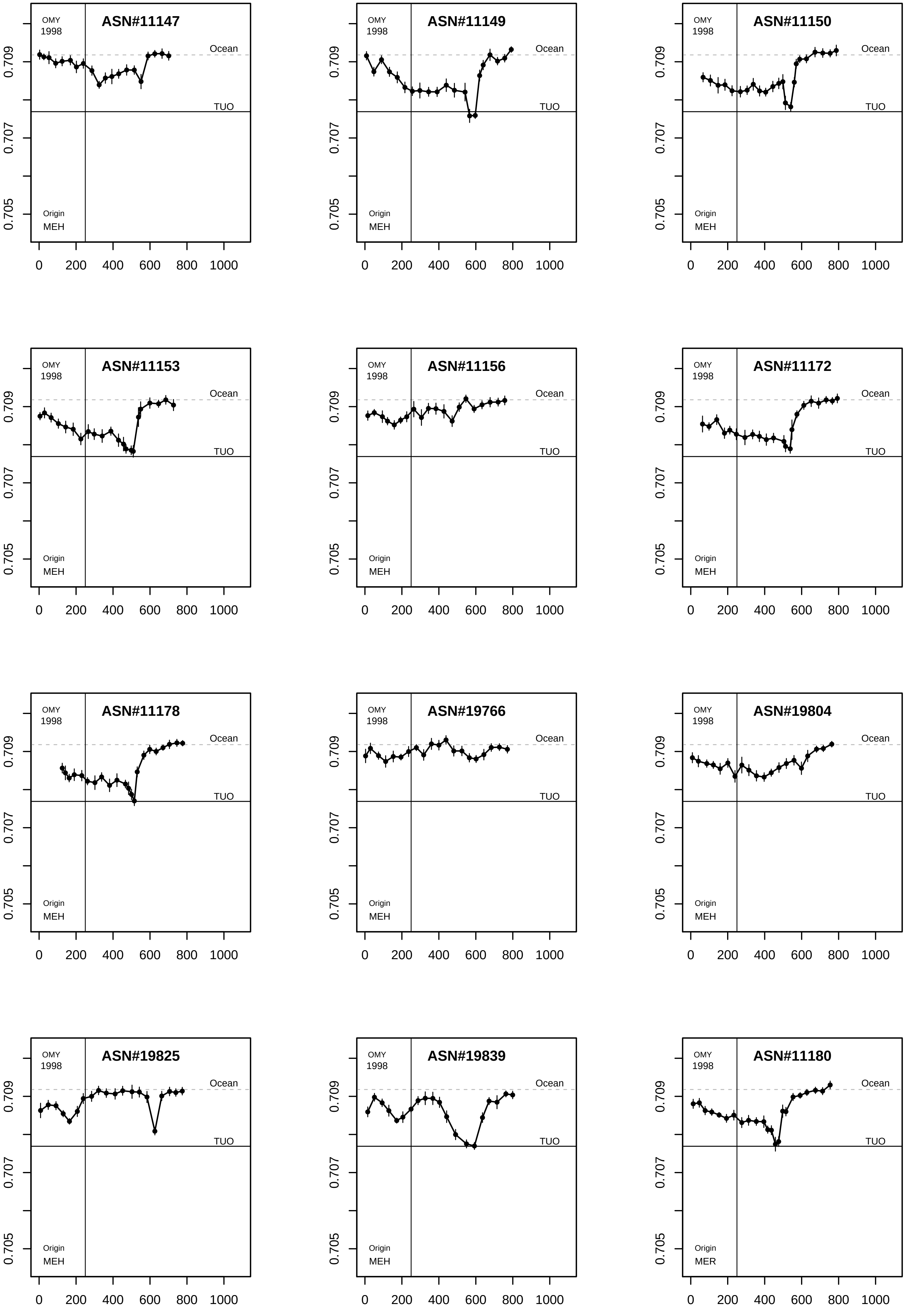
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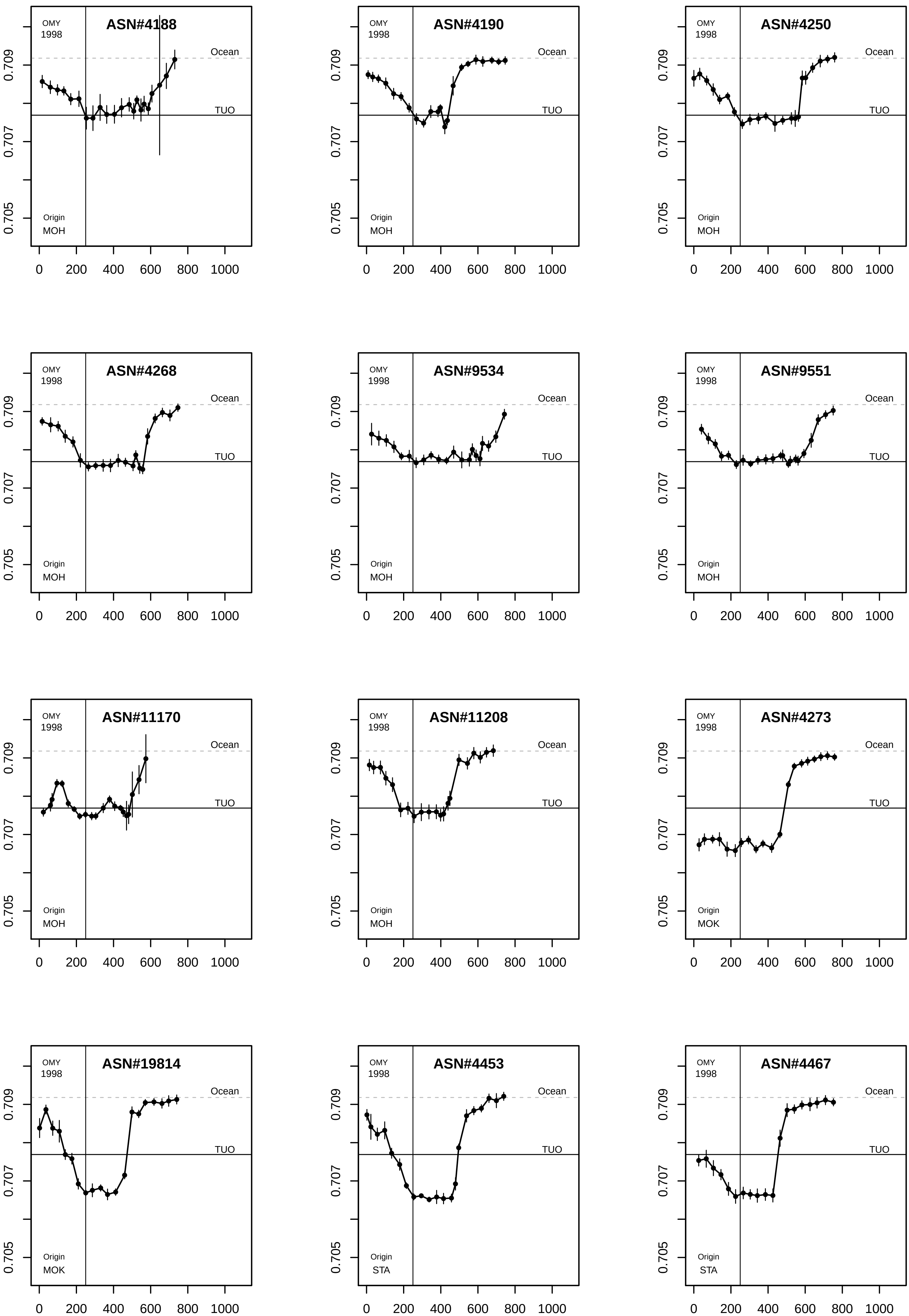
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ASN#4467
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Origin
MOH
MOK
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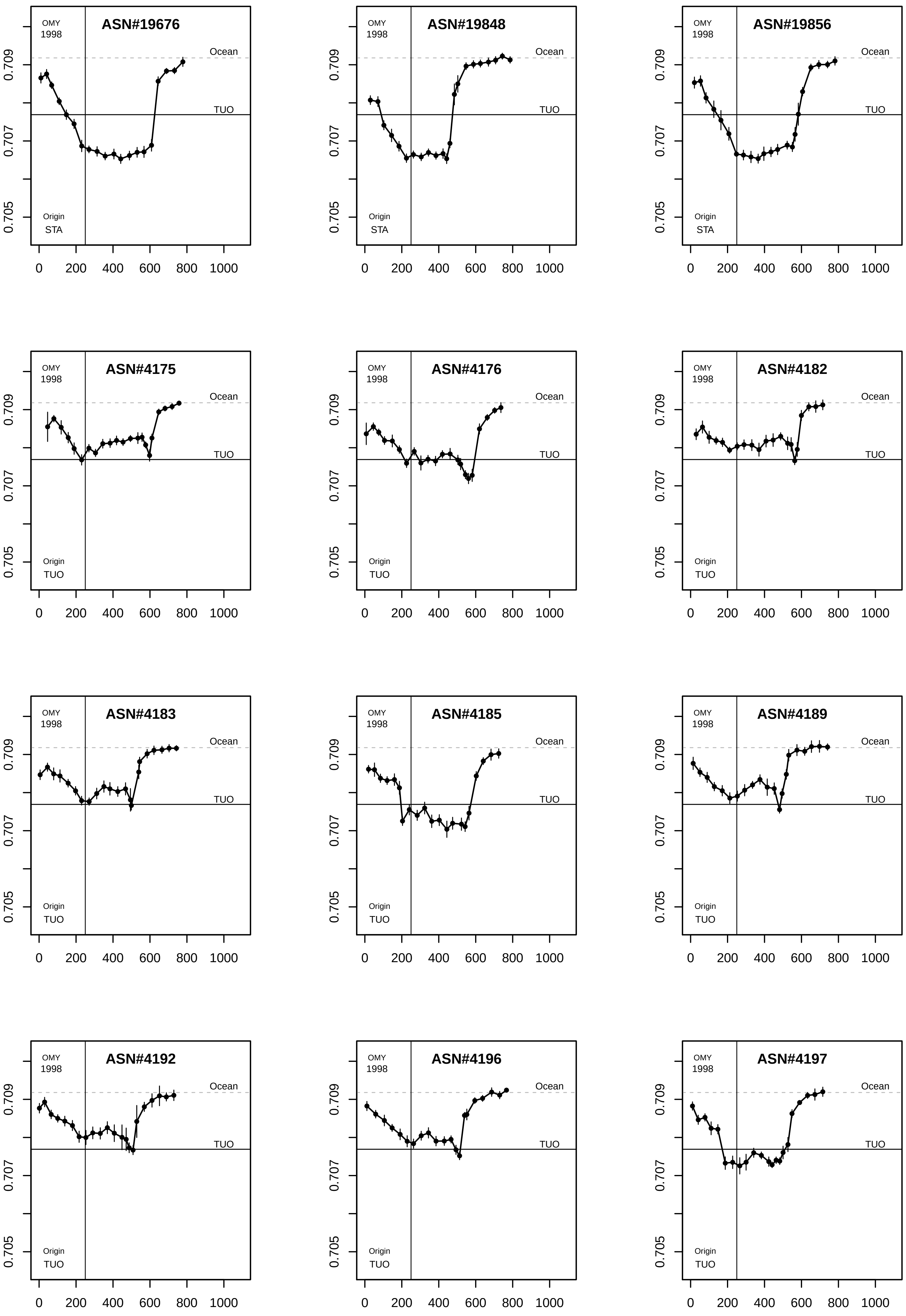

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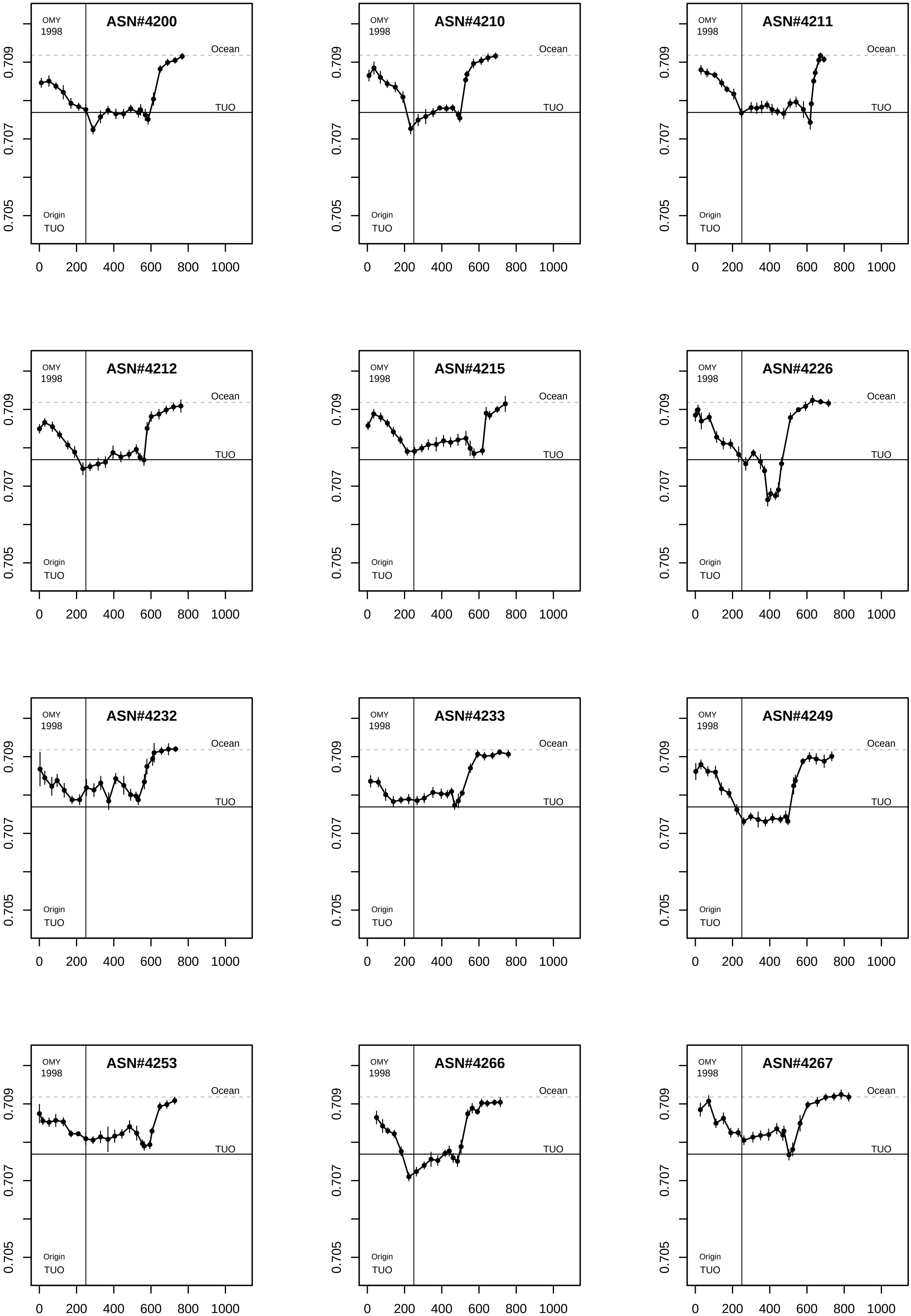
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TUO
Origin
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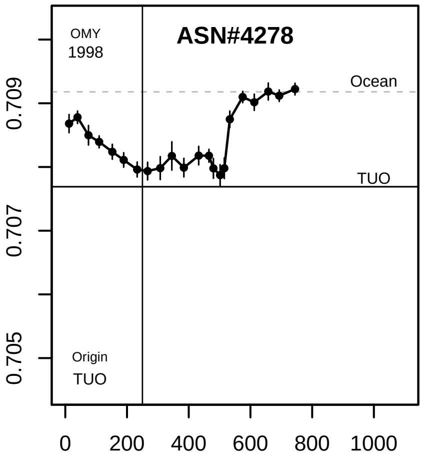
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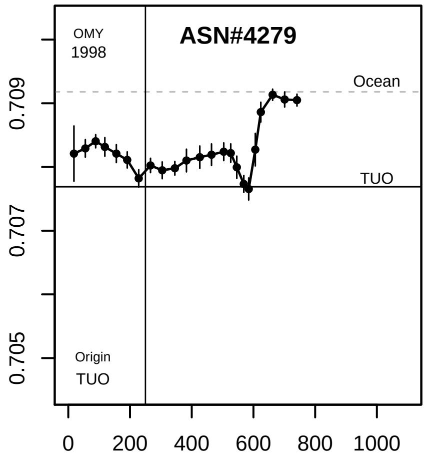
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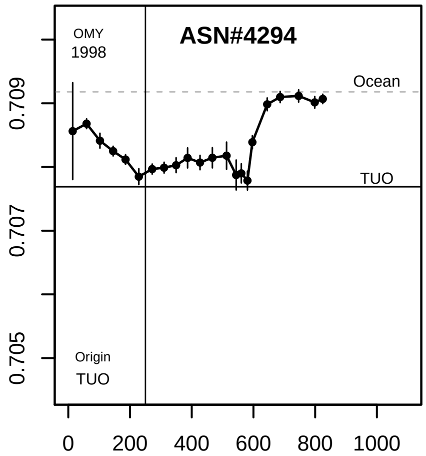
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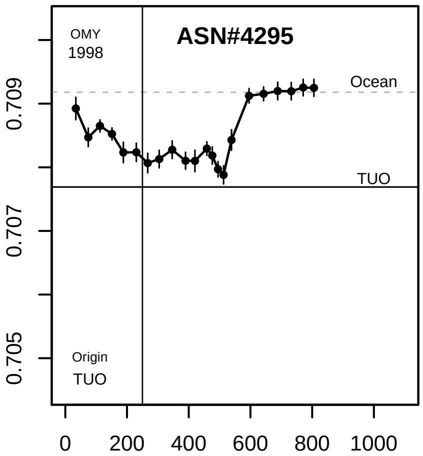
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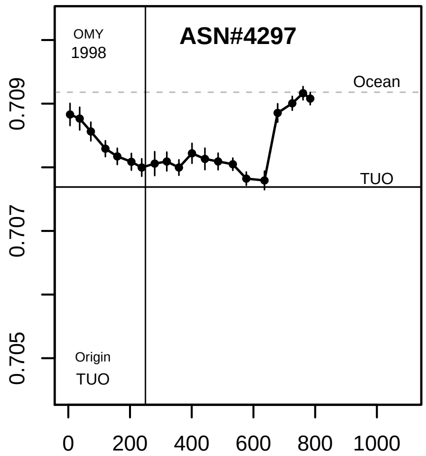
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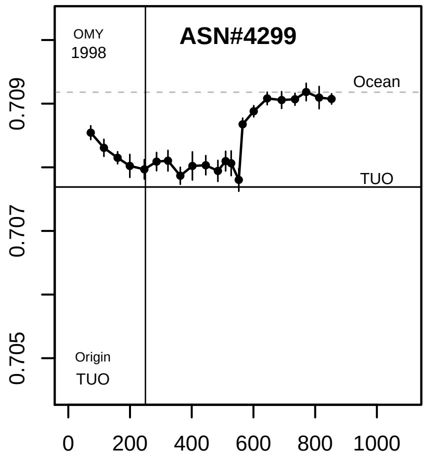
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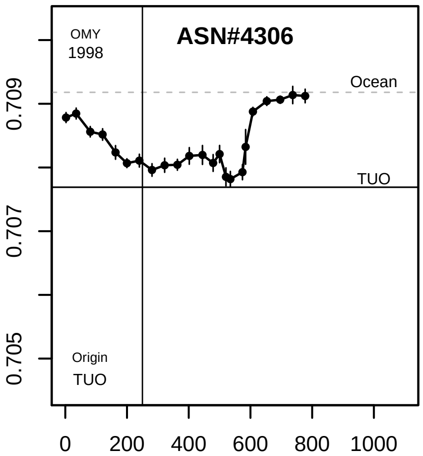
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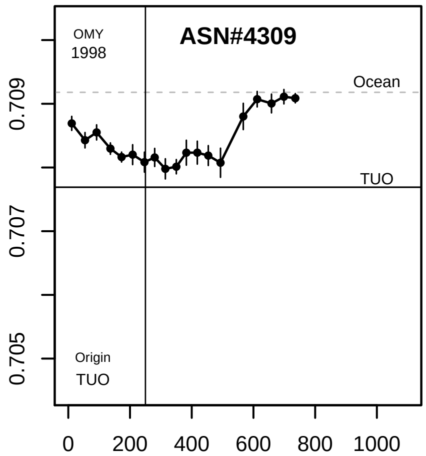
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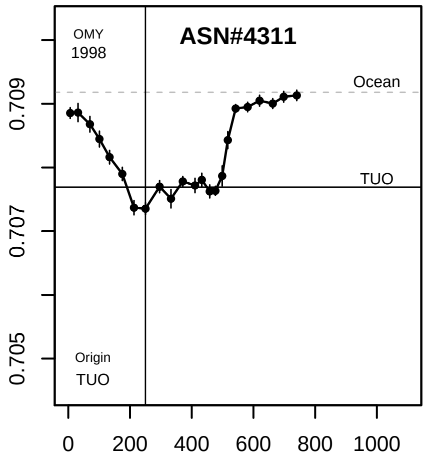
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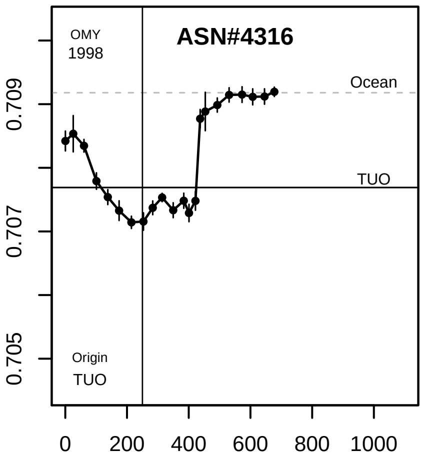
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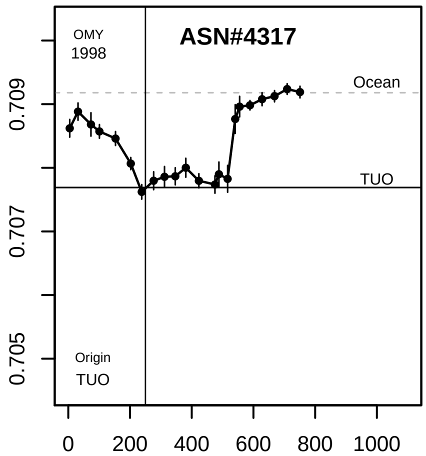
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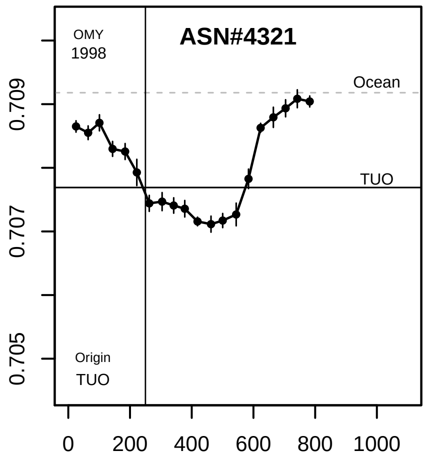
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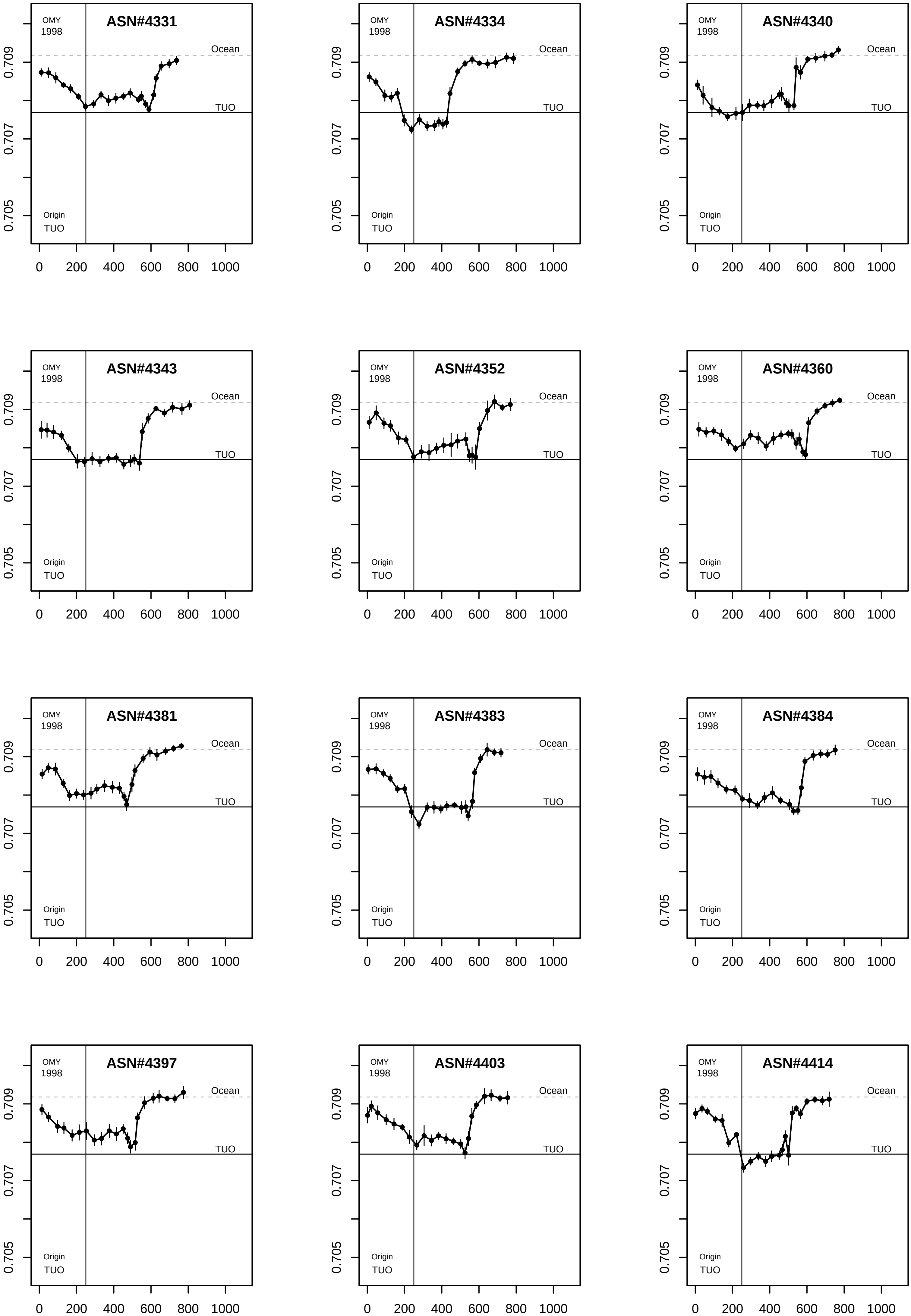

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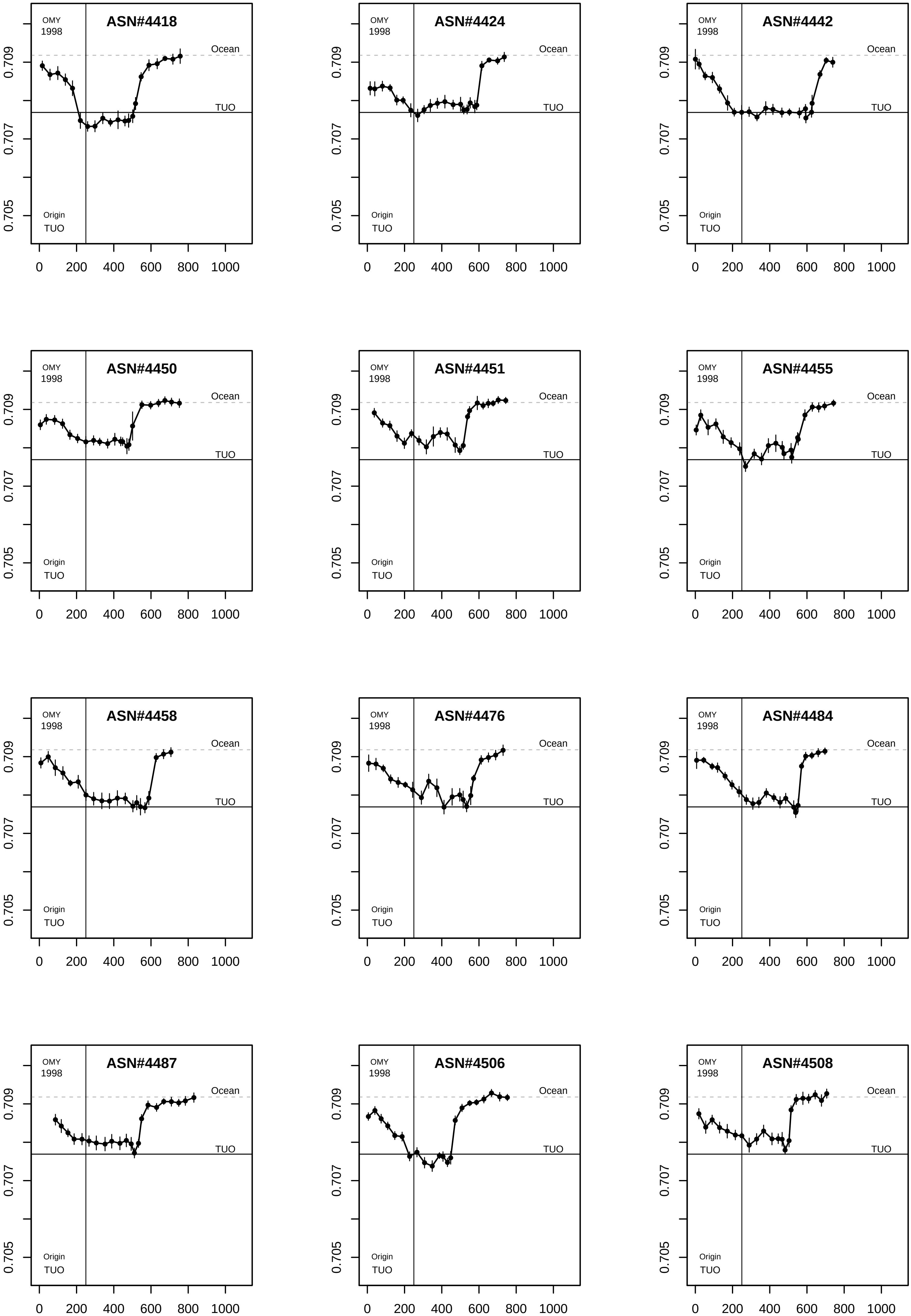
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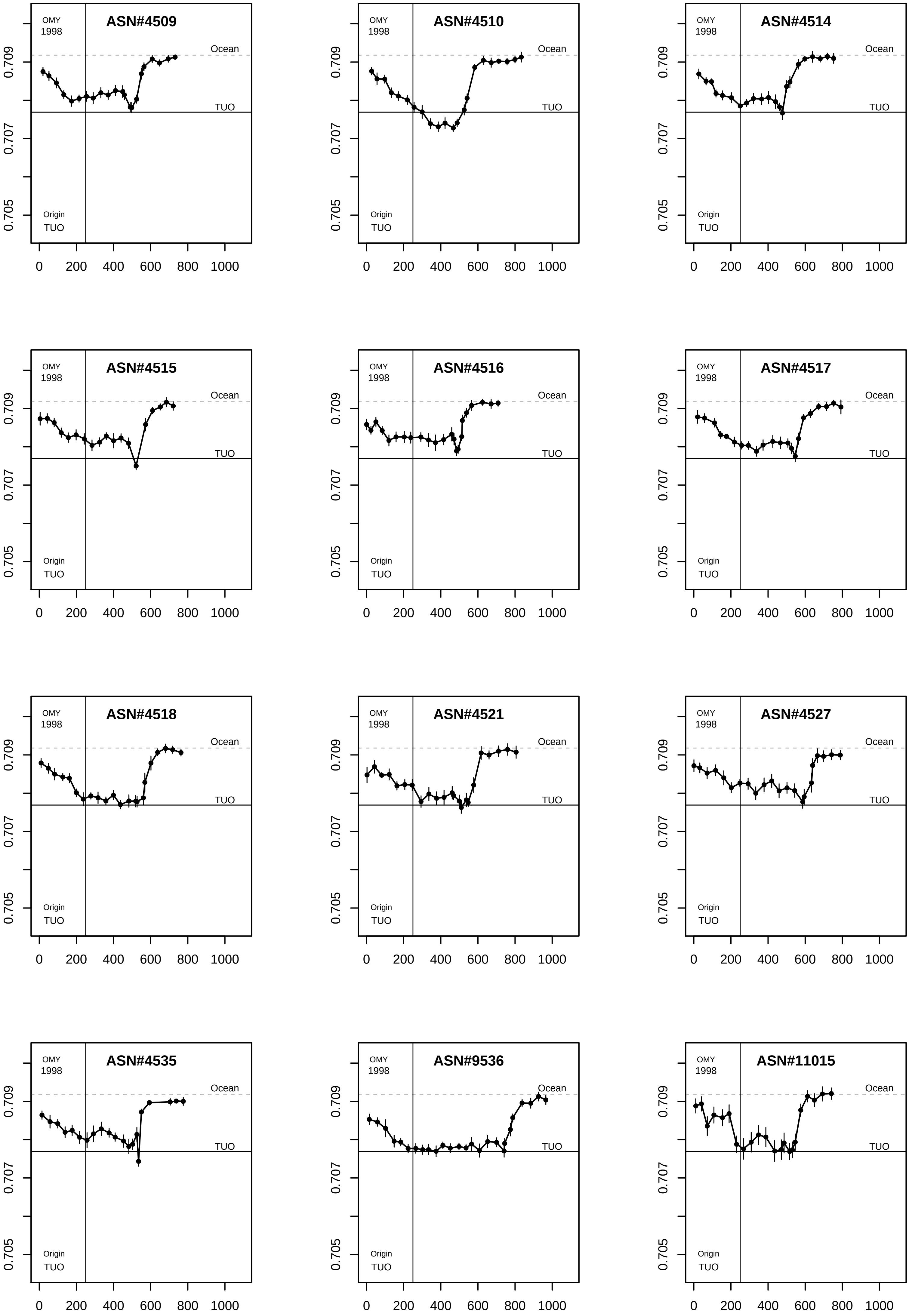
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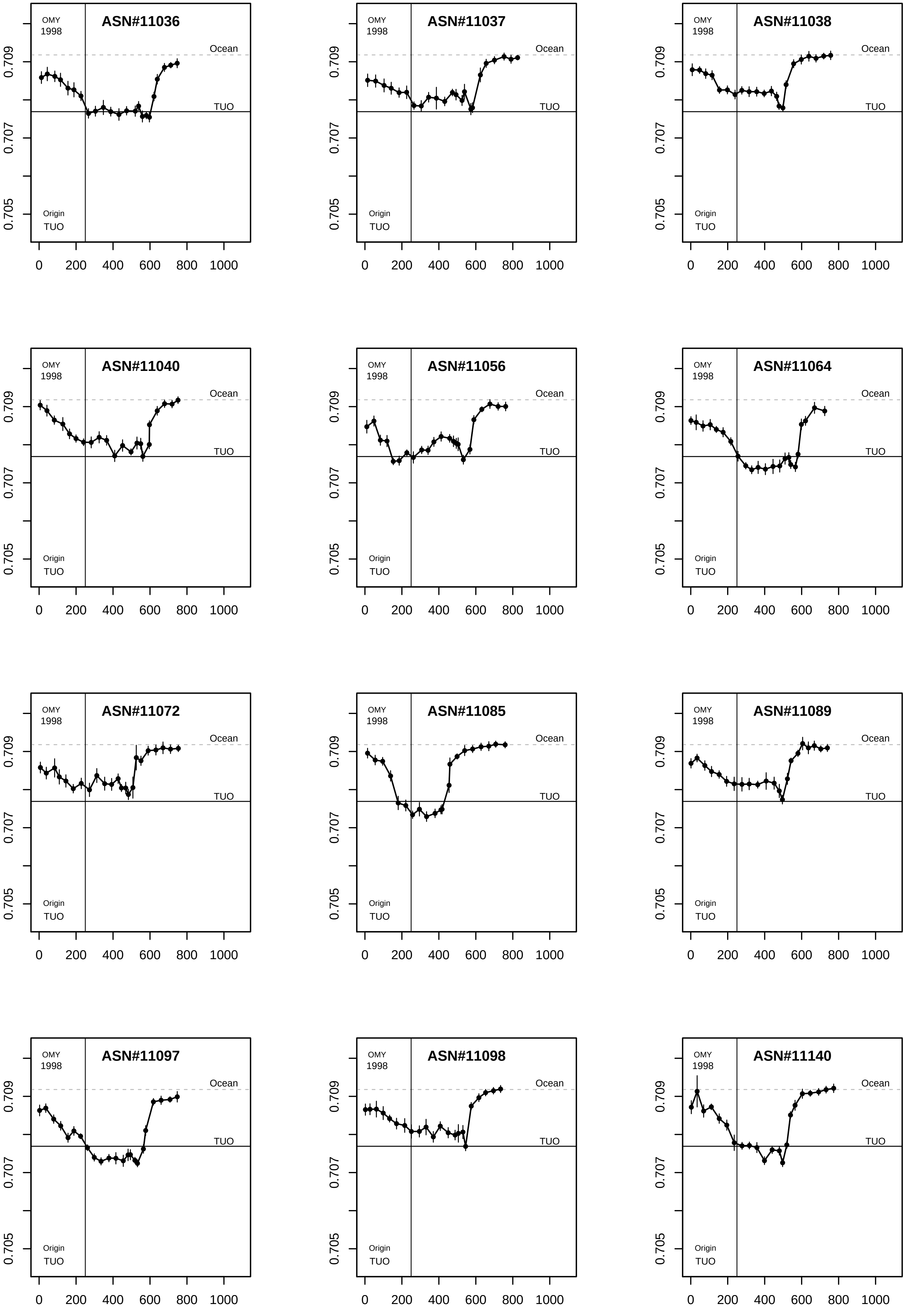
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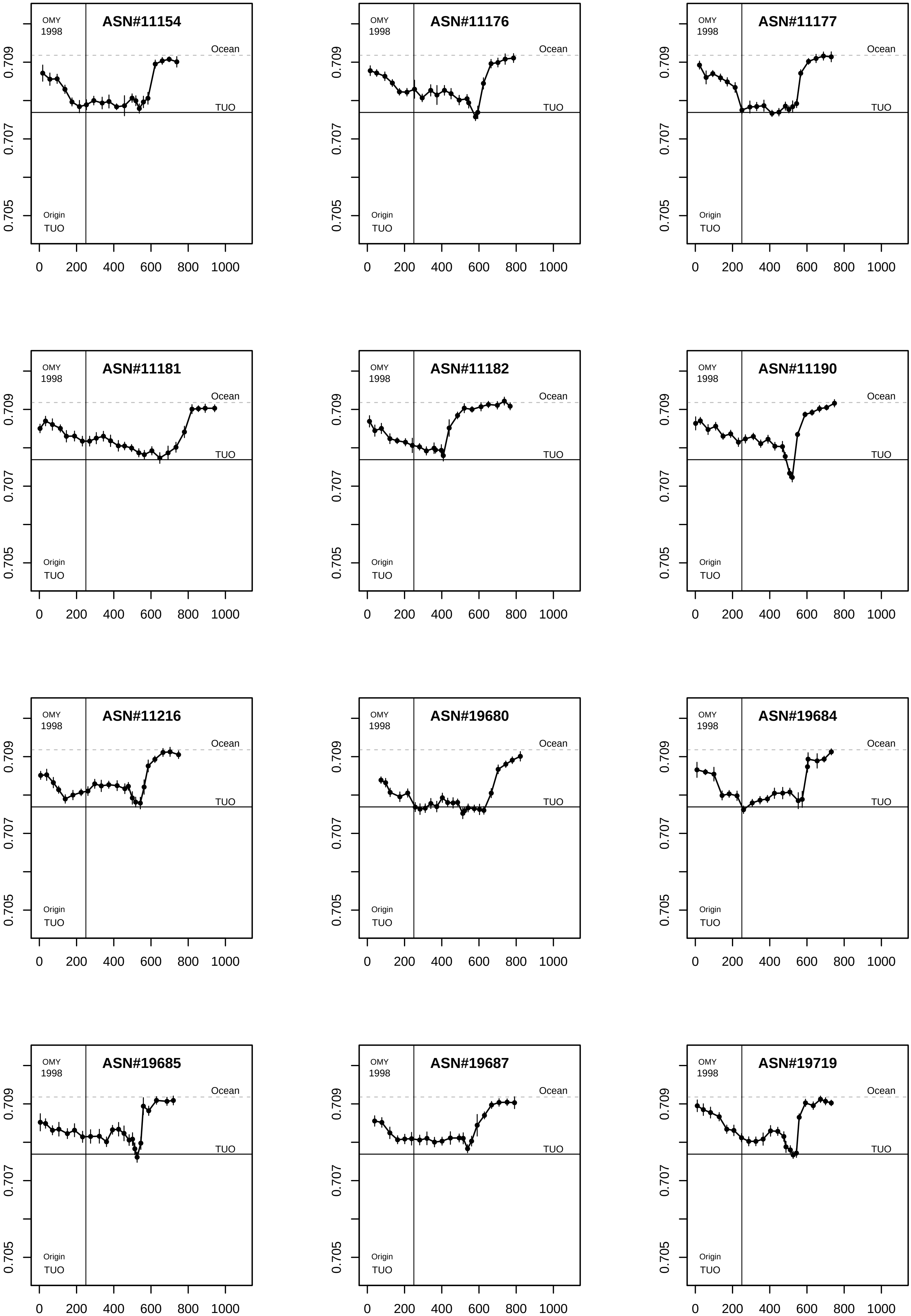
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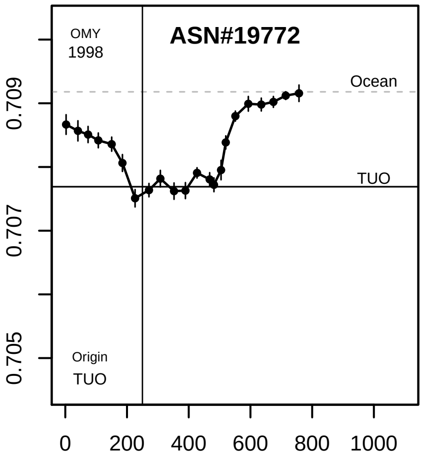
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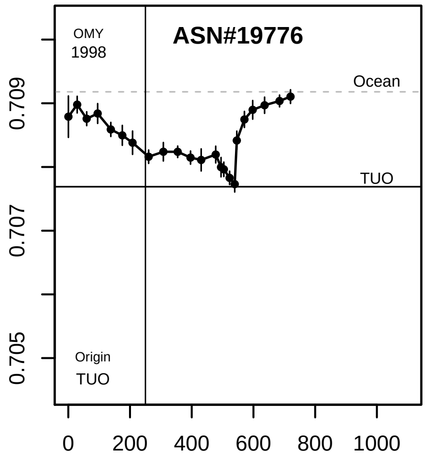
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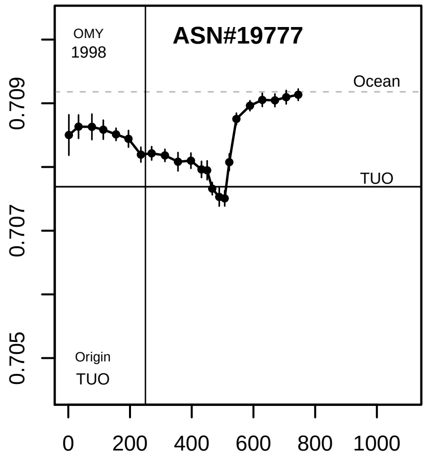
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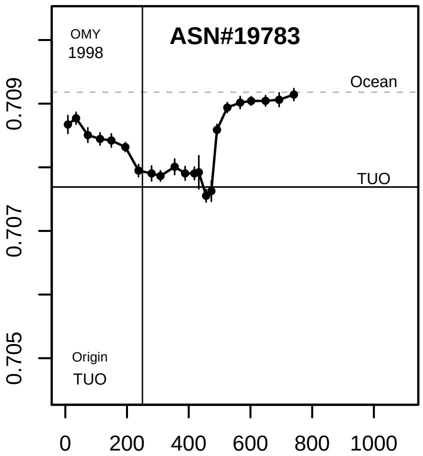
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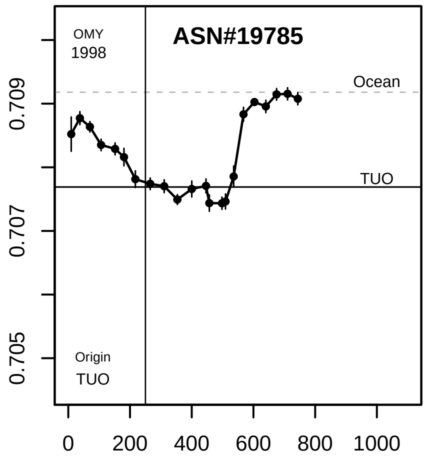
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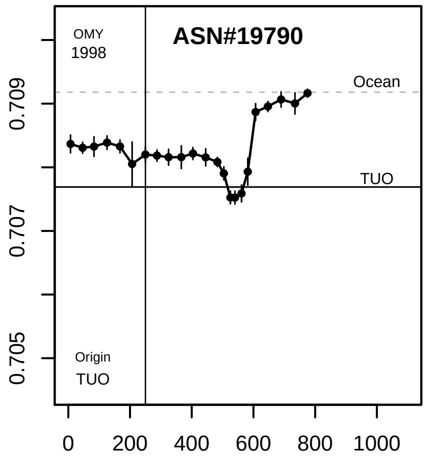
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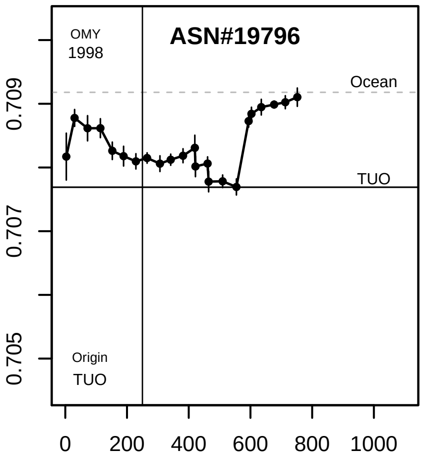
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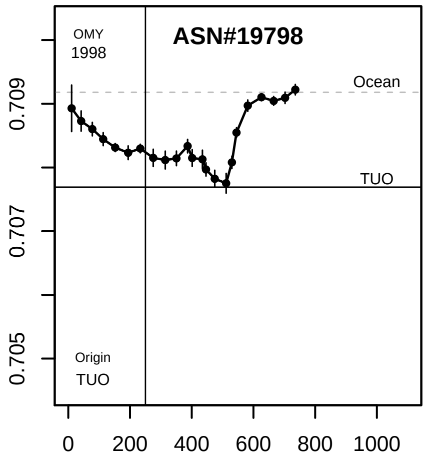
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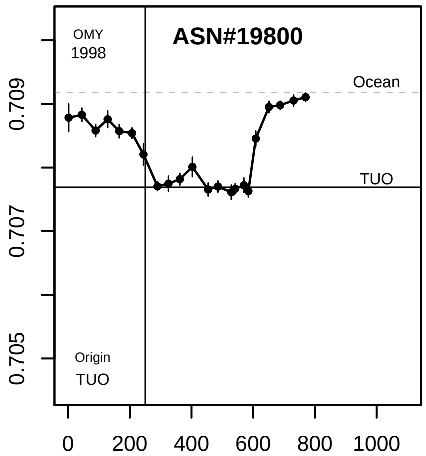
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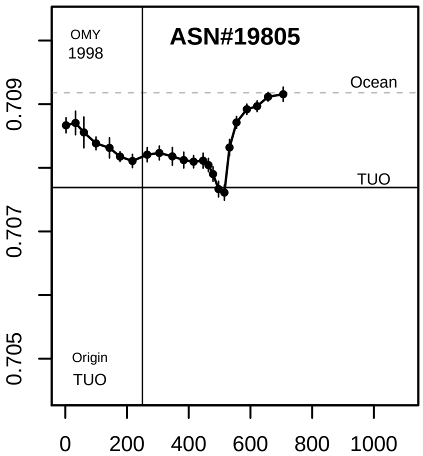
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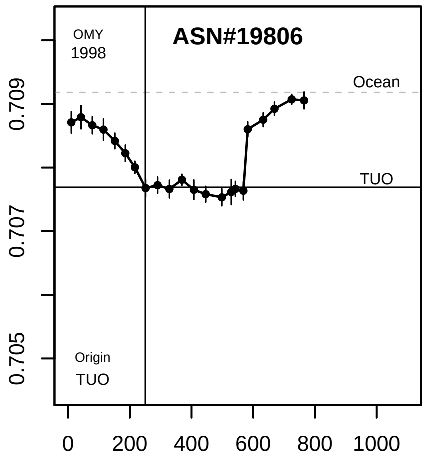
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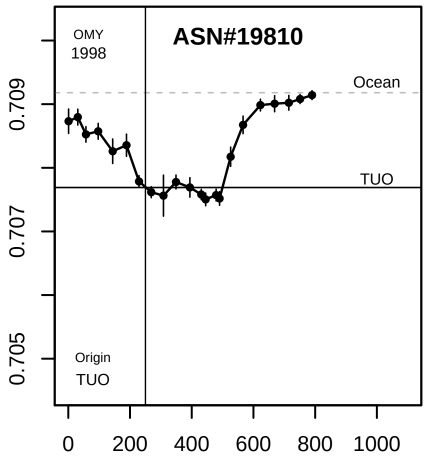
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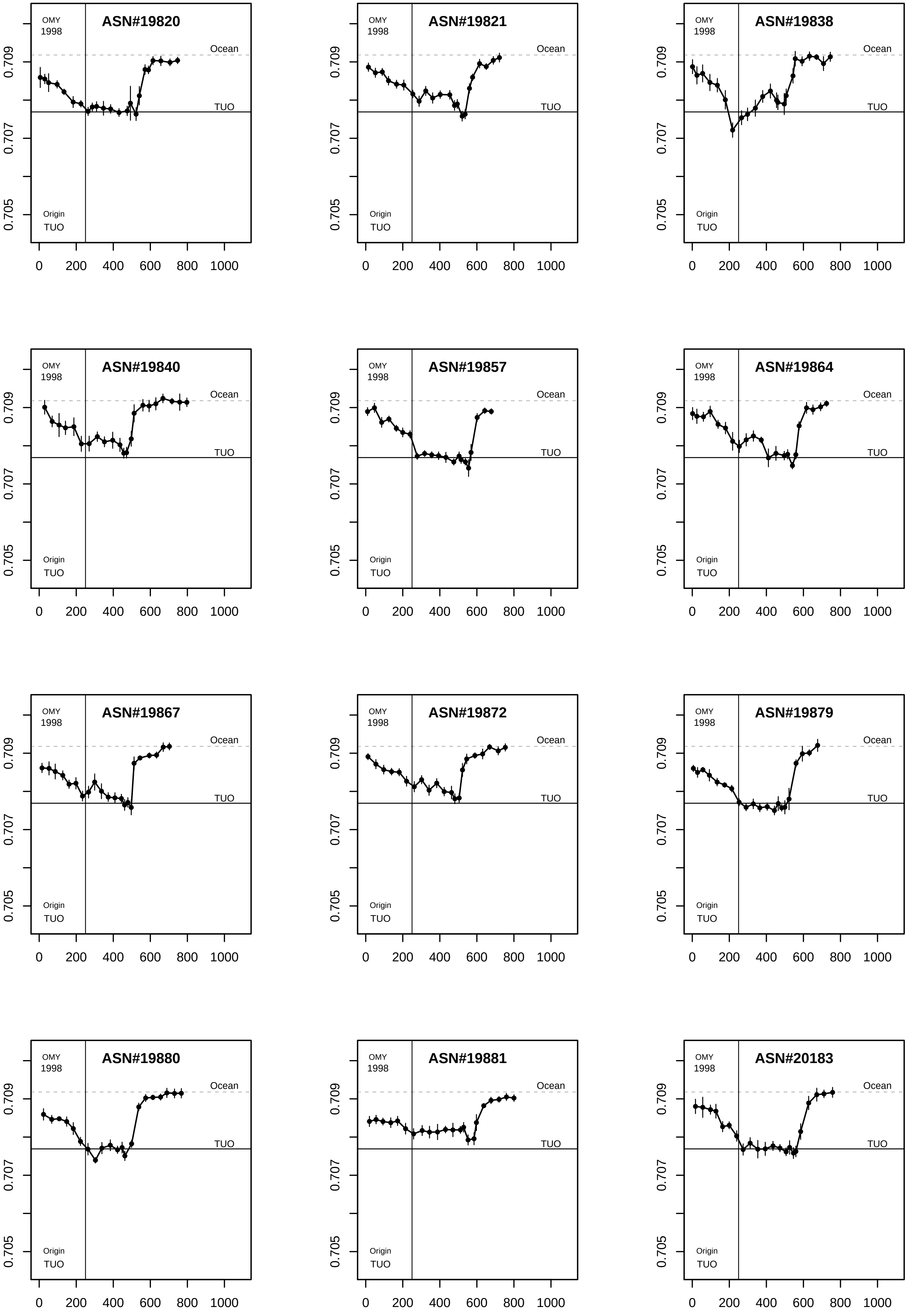

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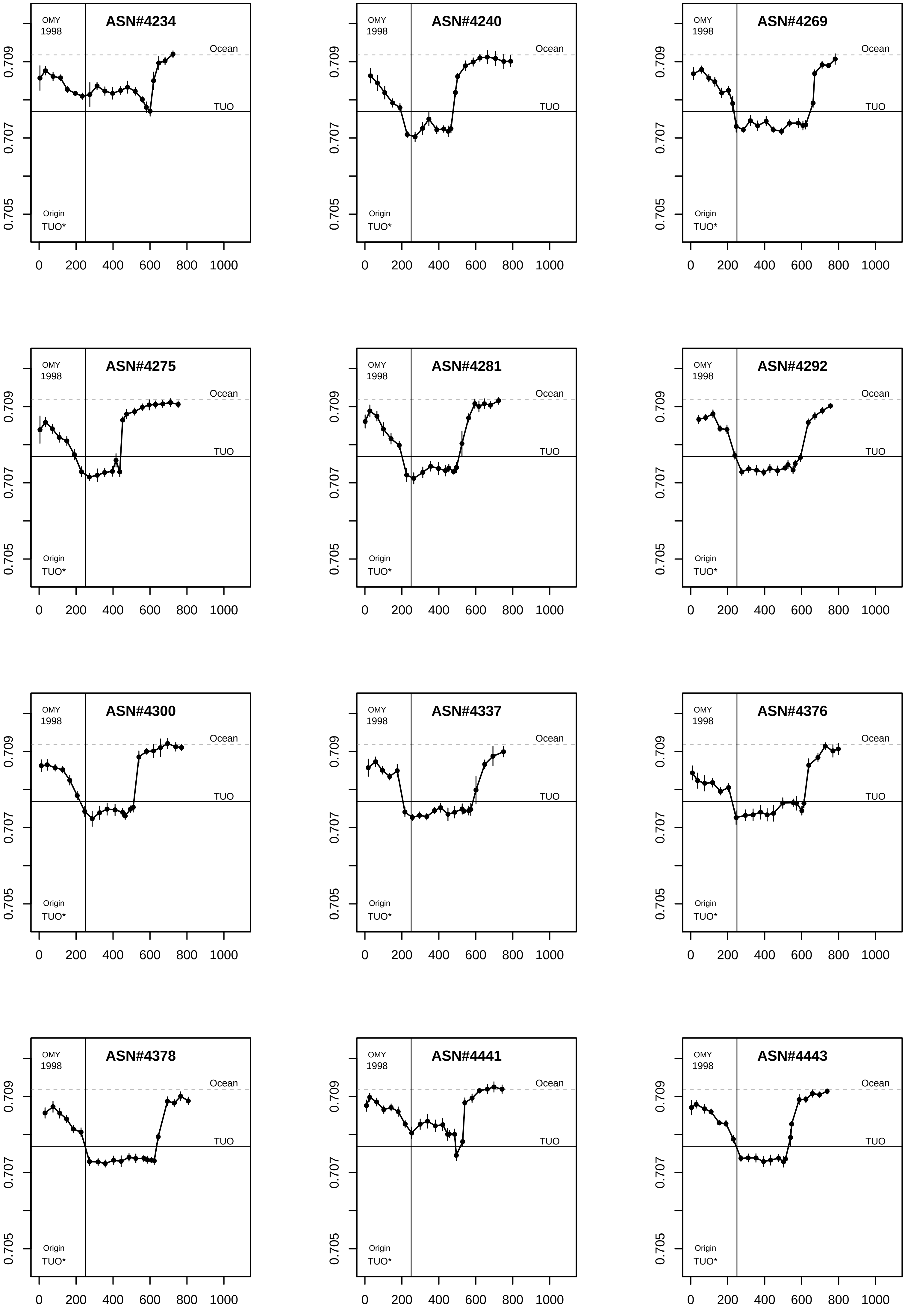
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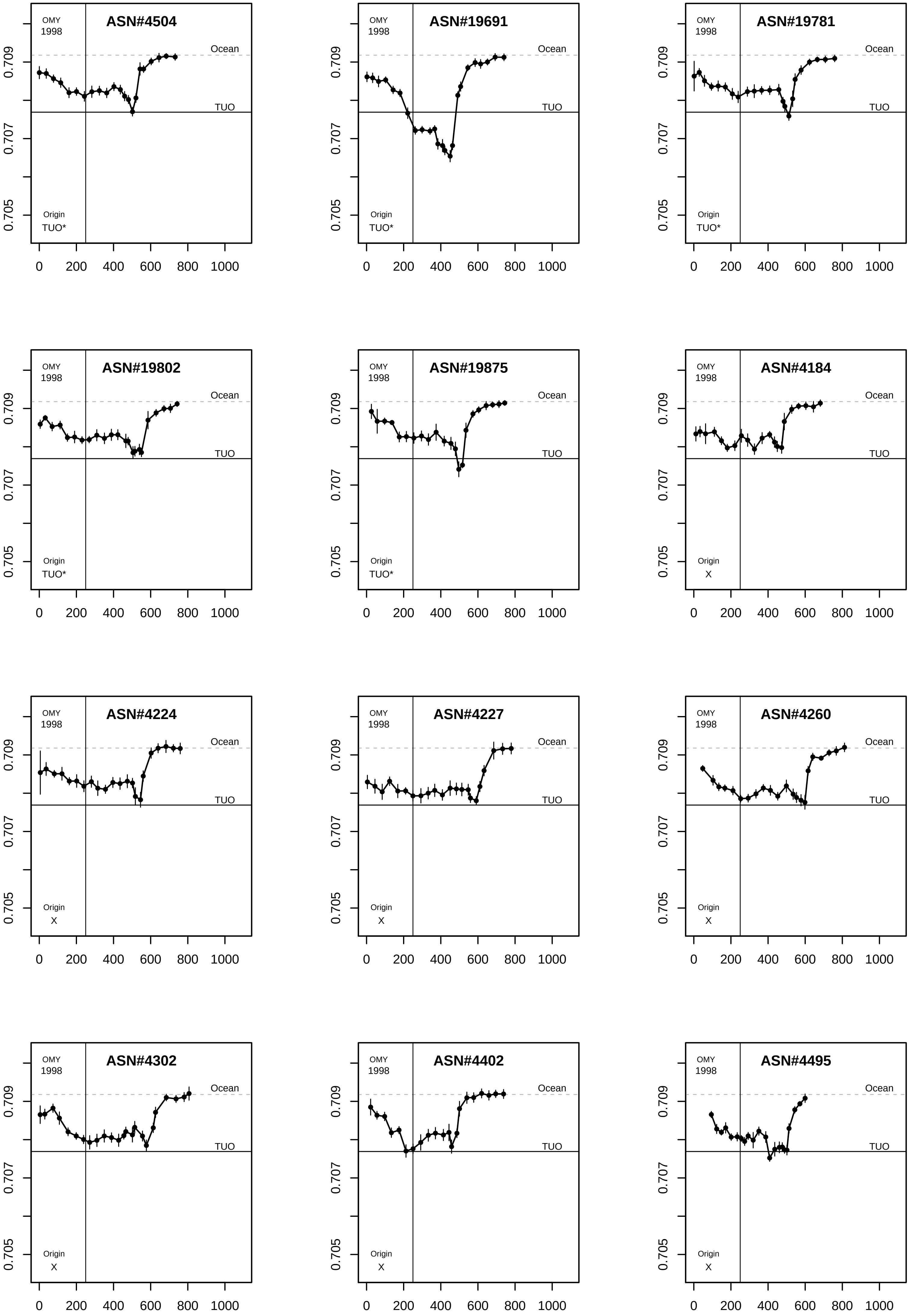

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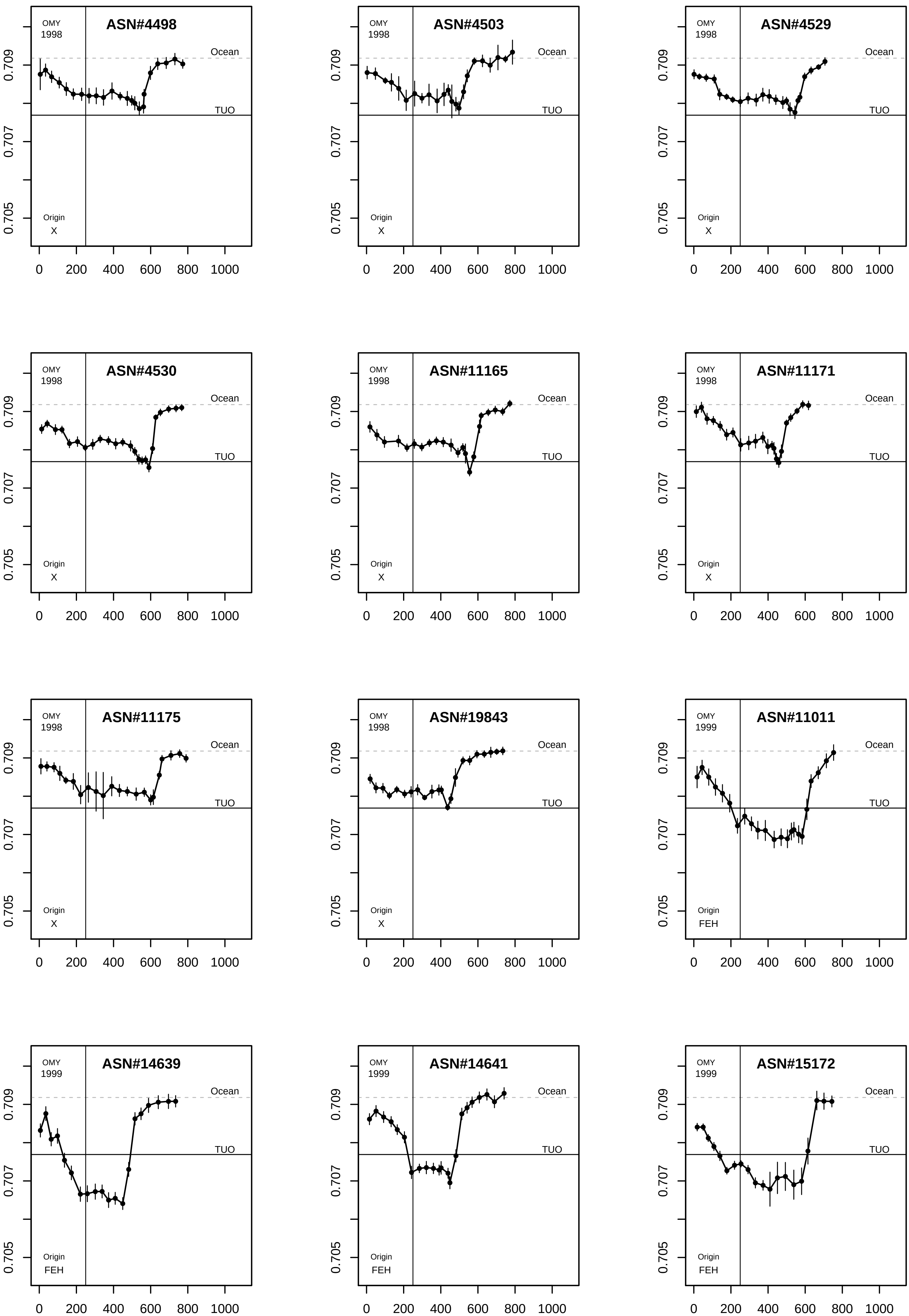
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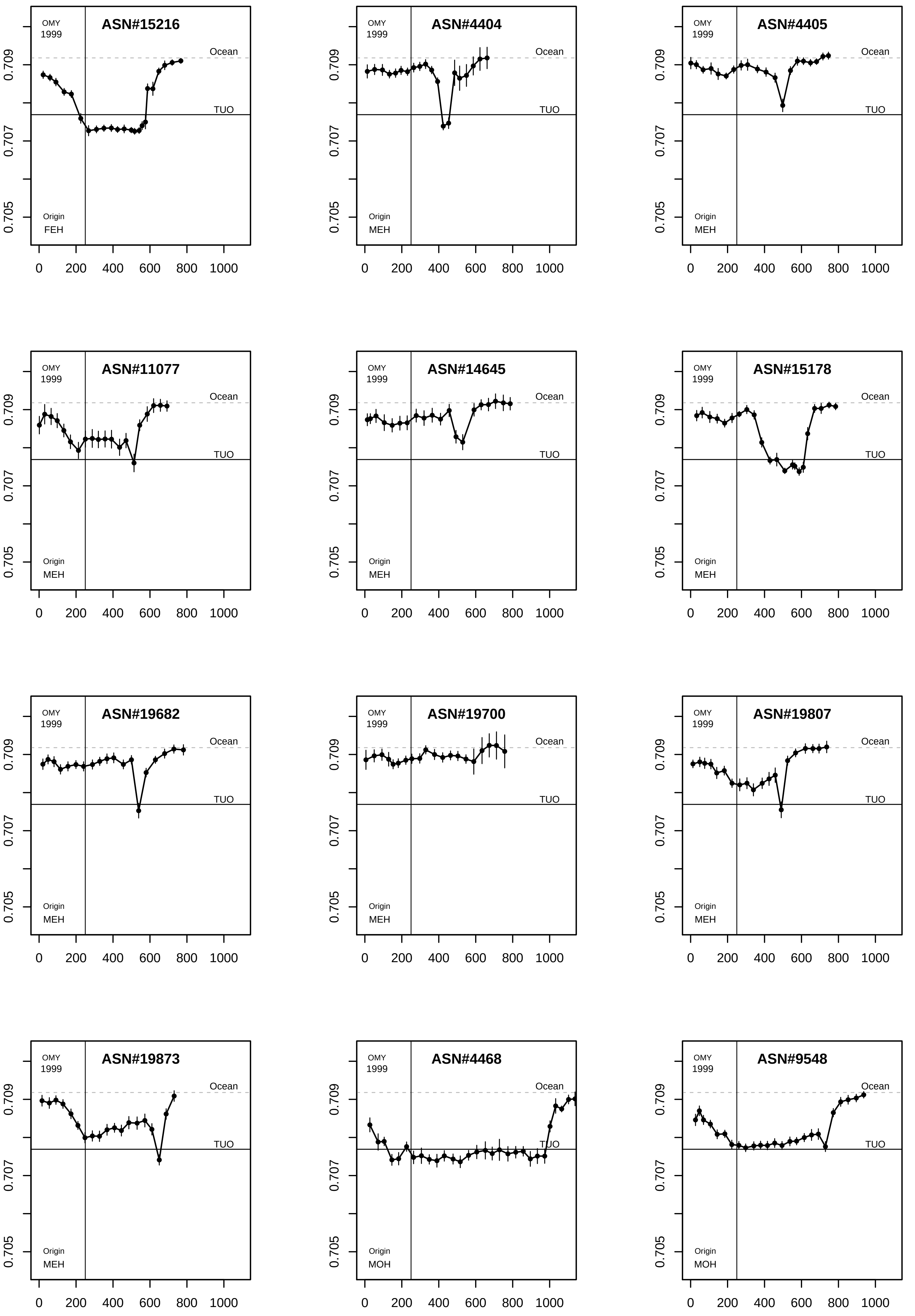
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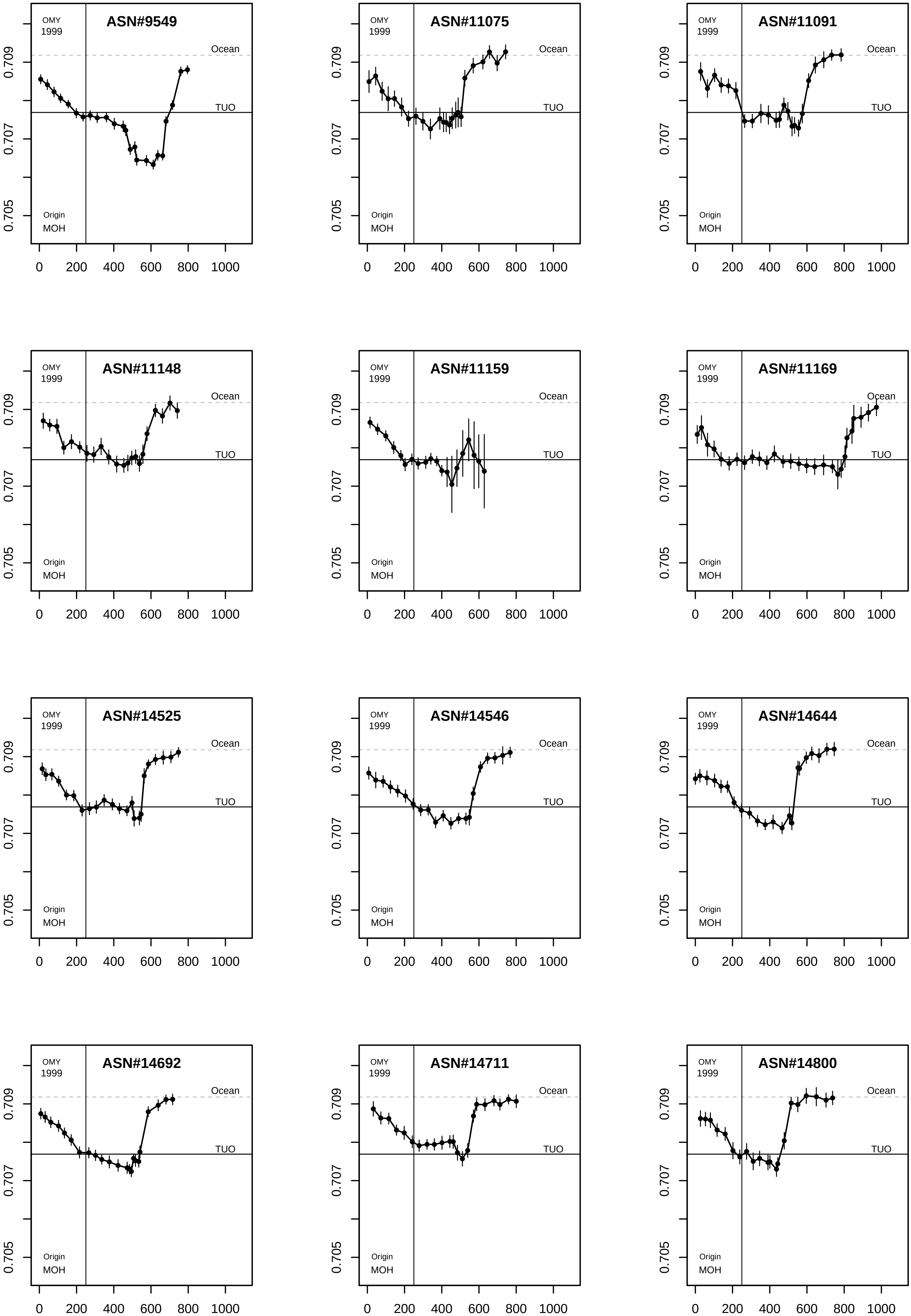
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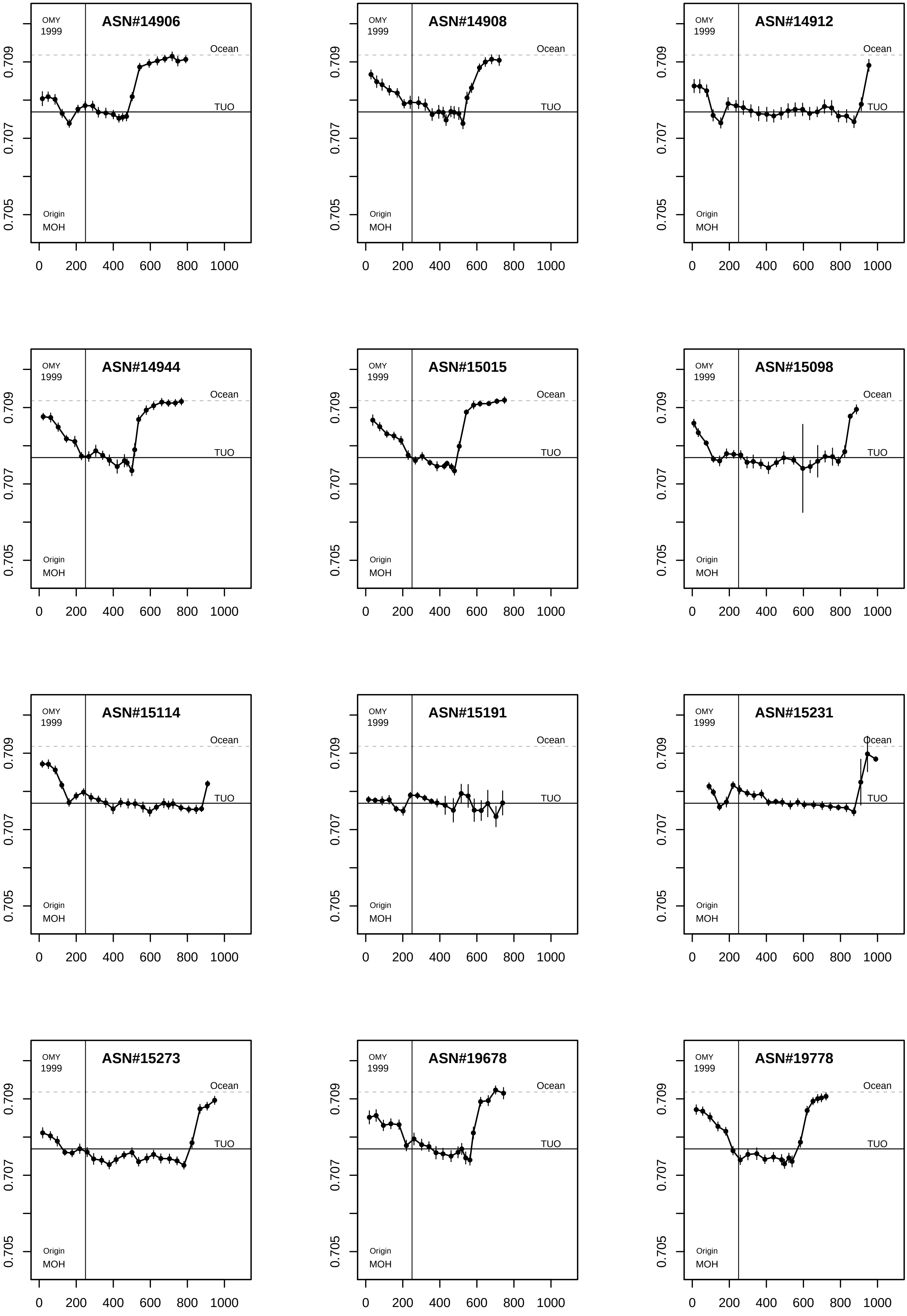
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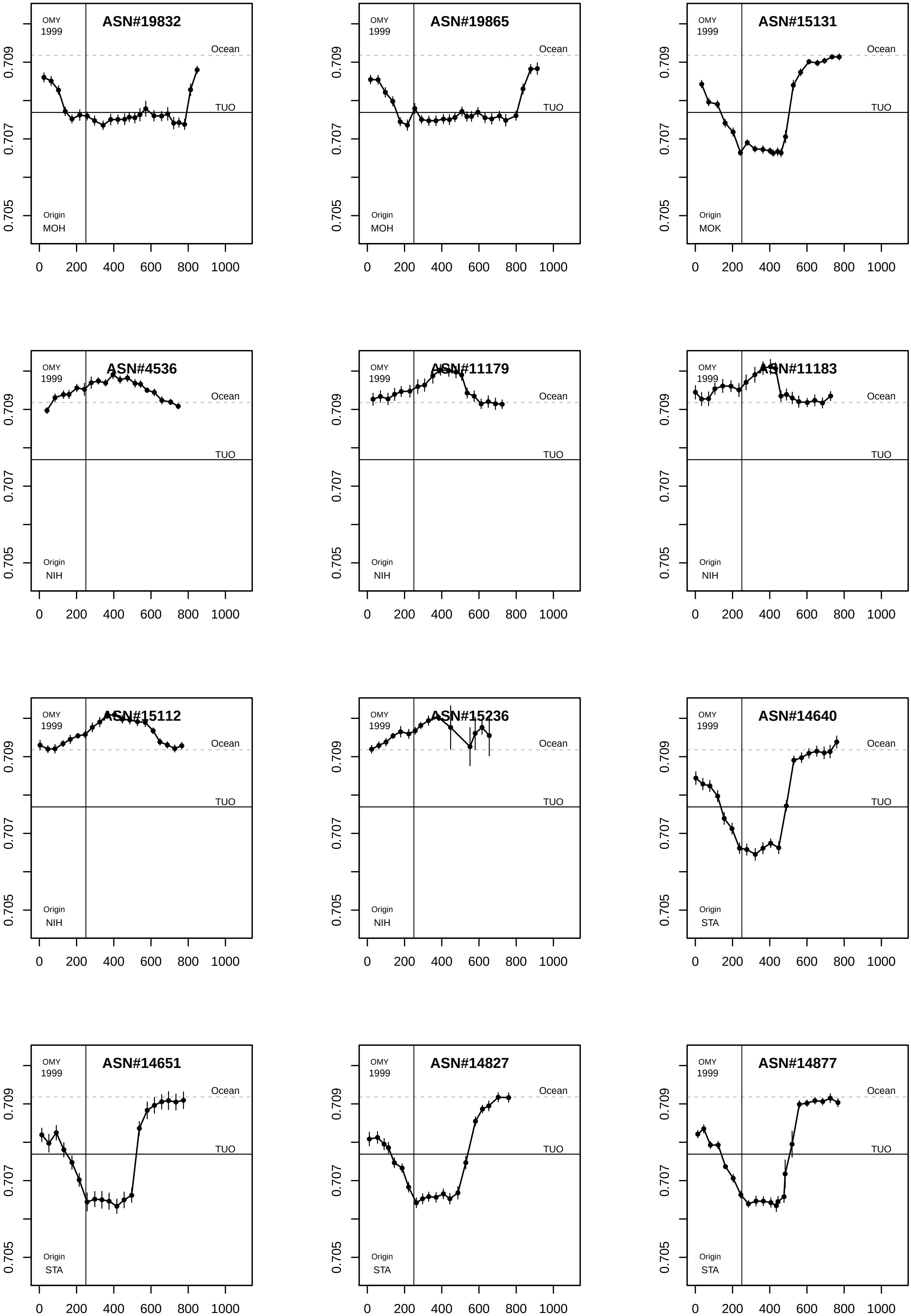

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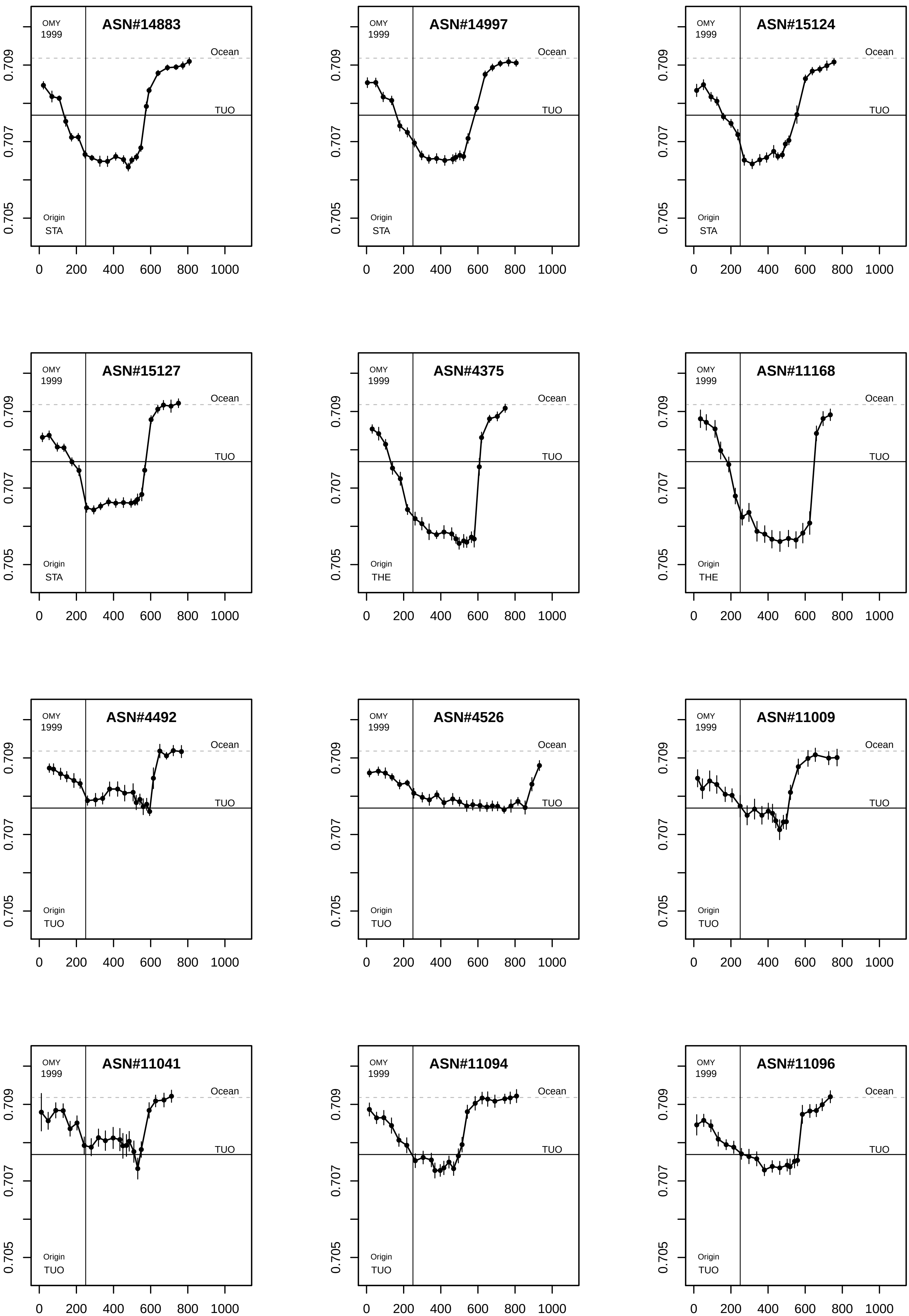

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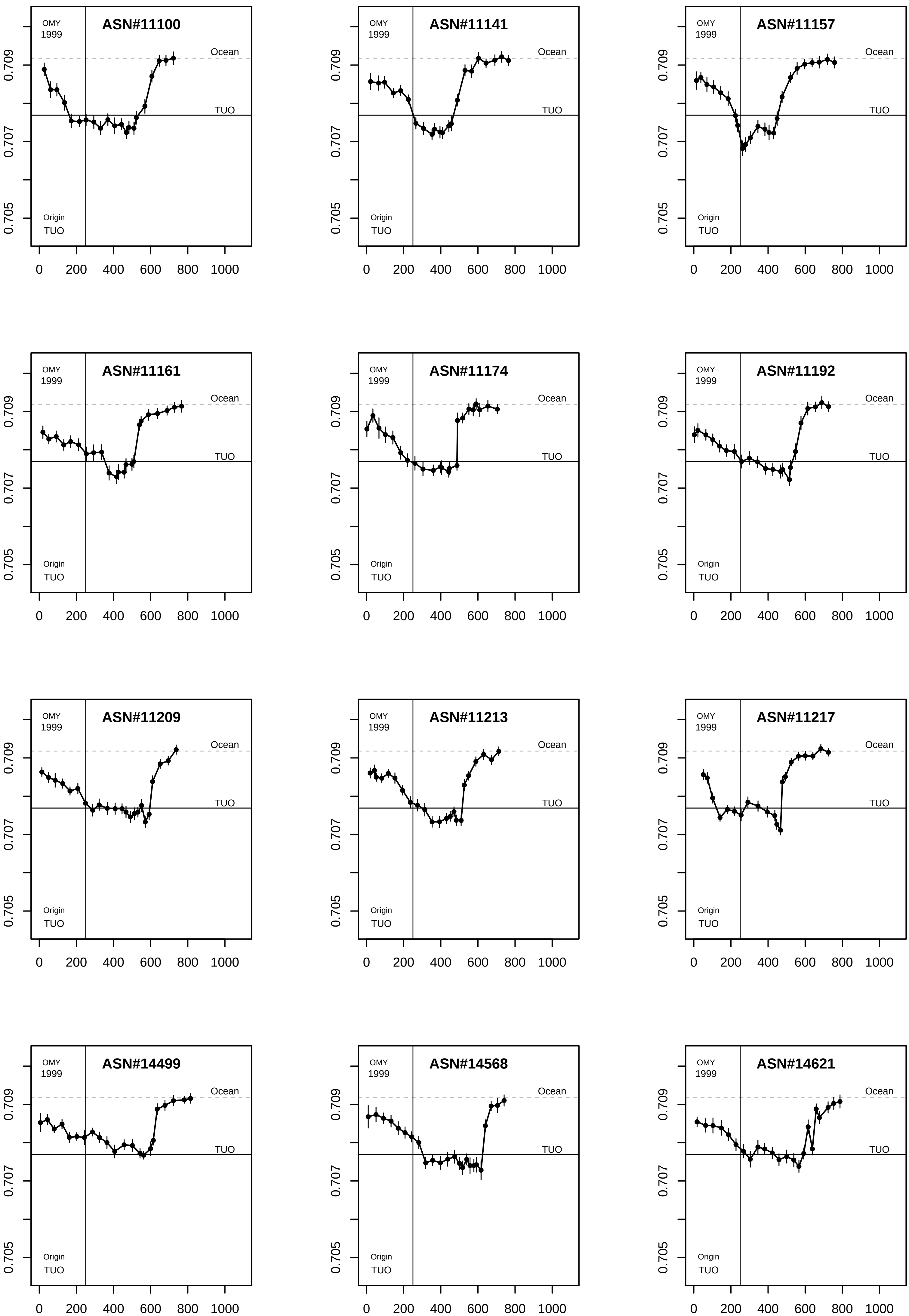
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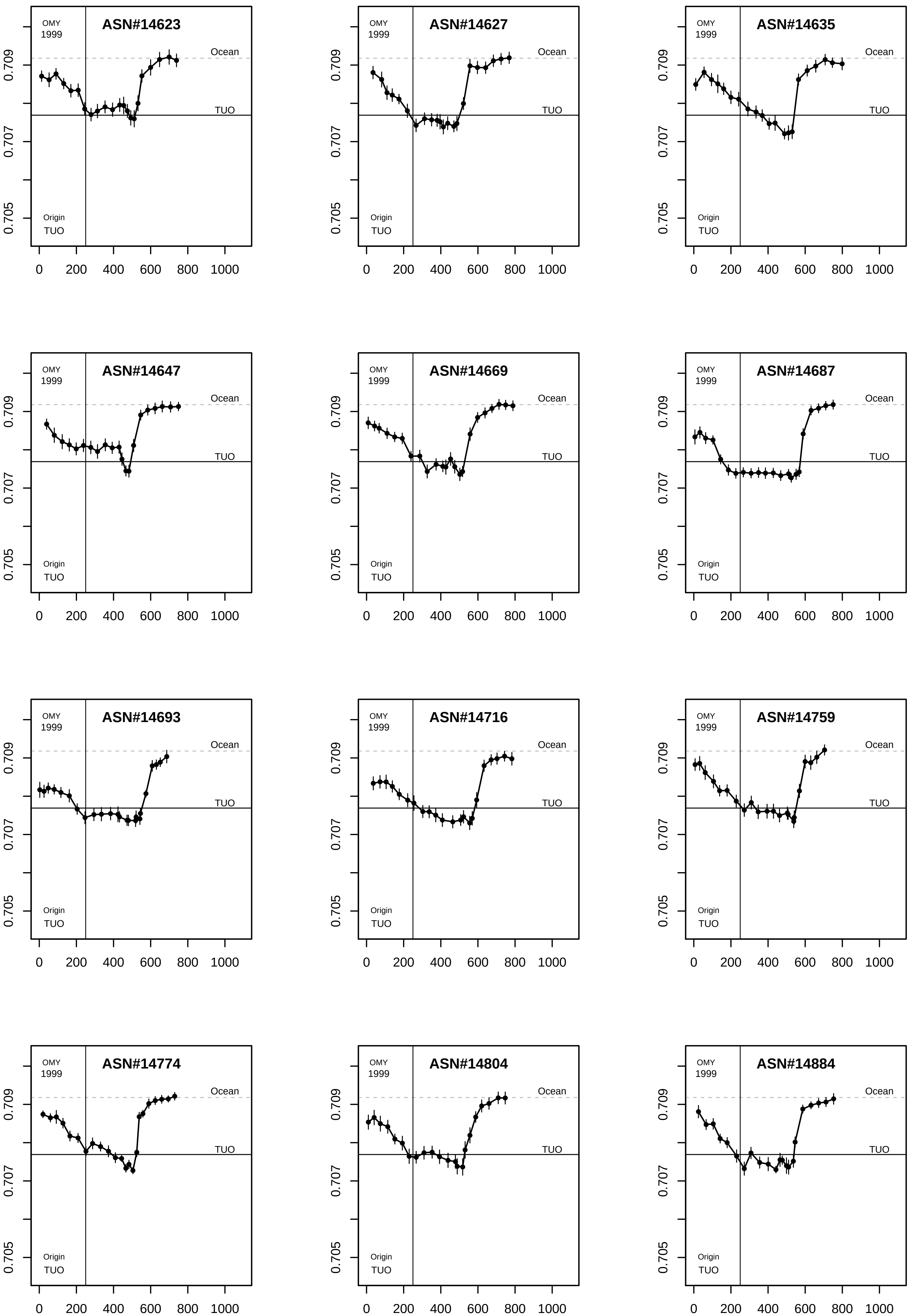

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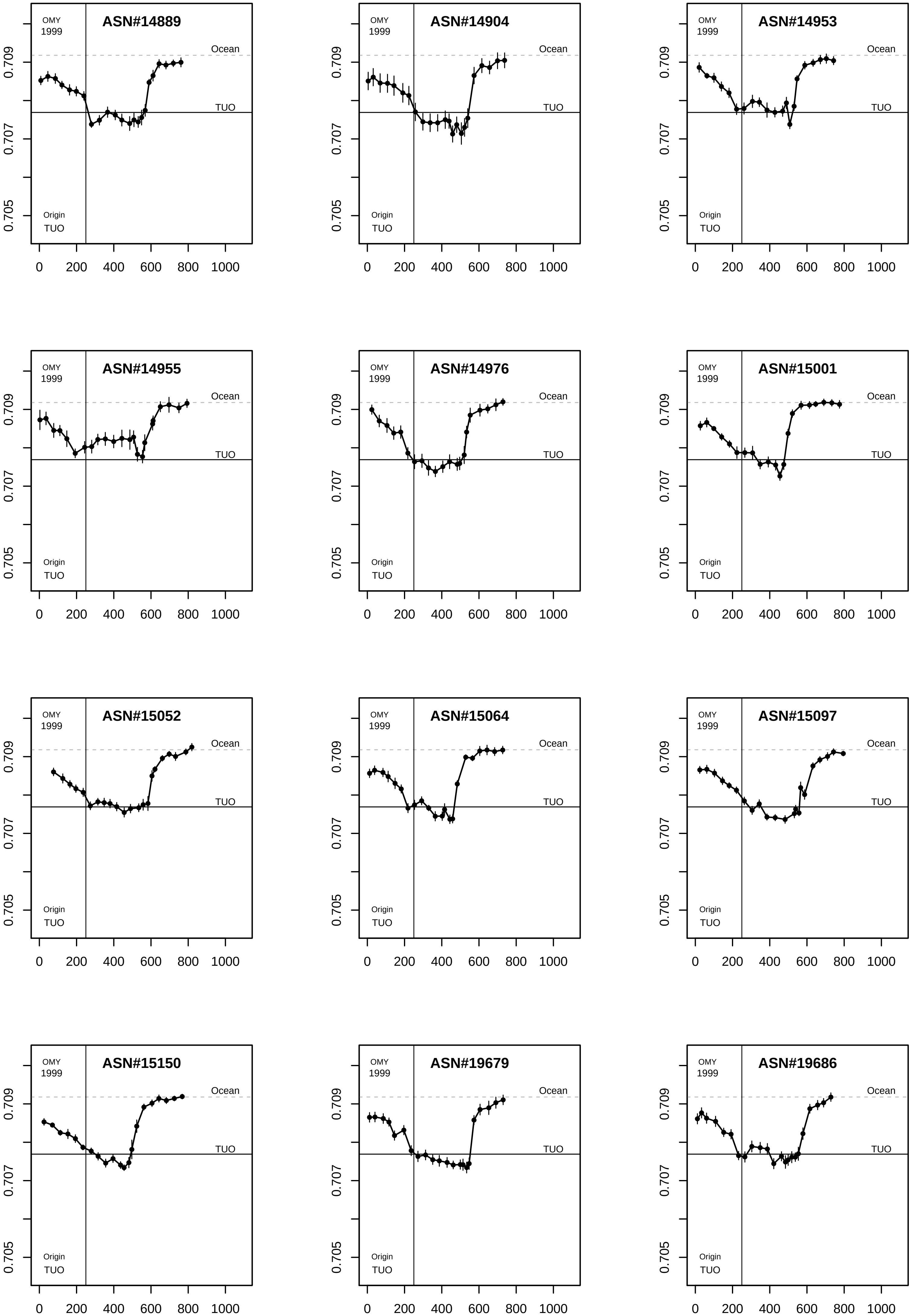
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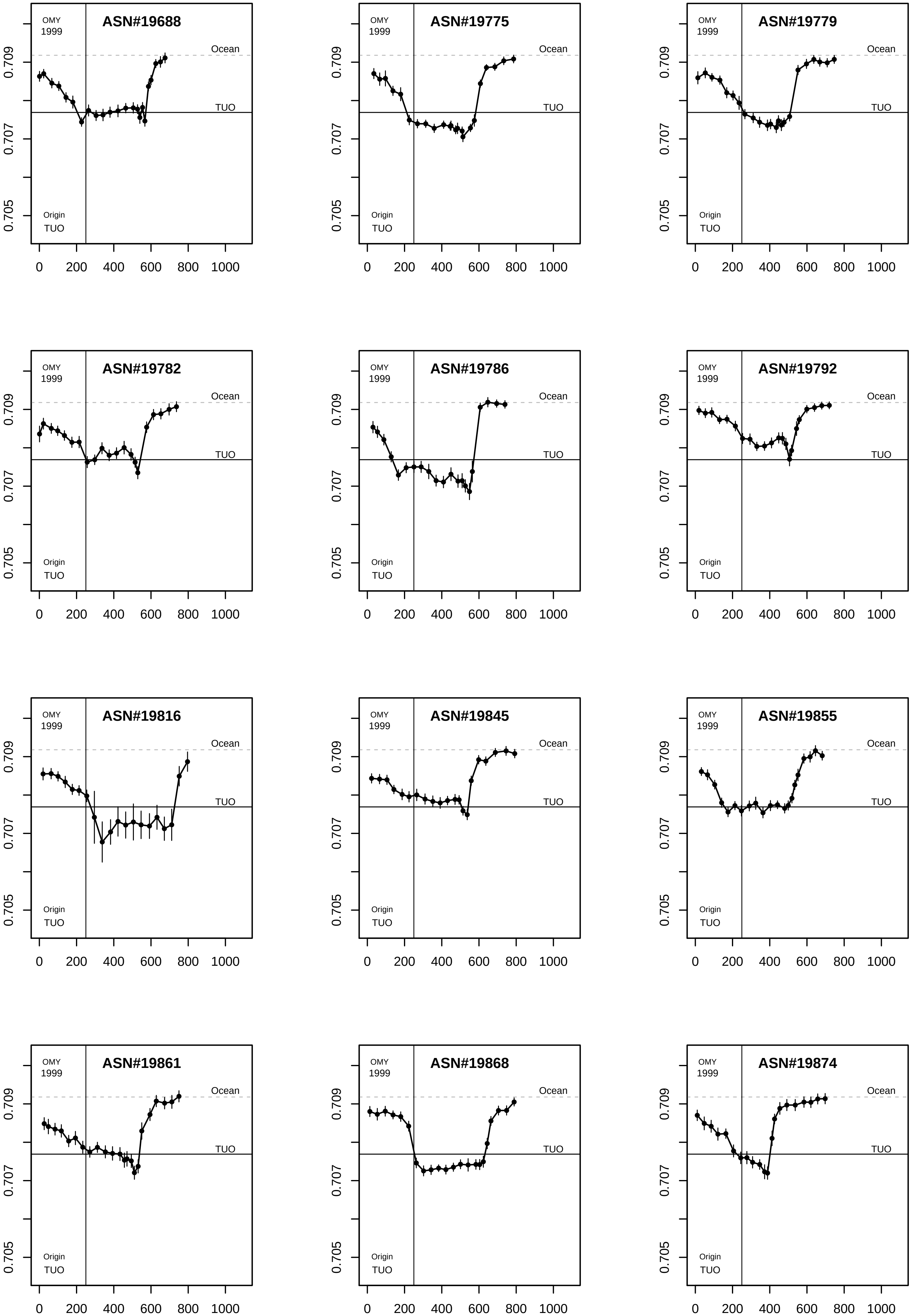
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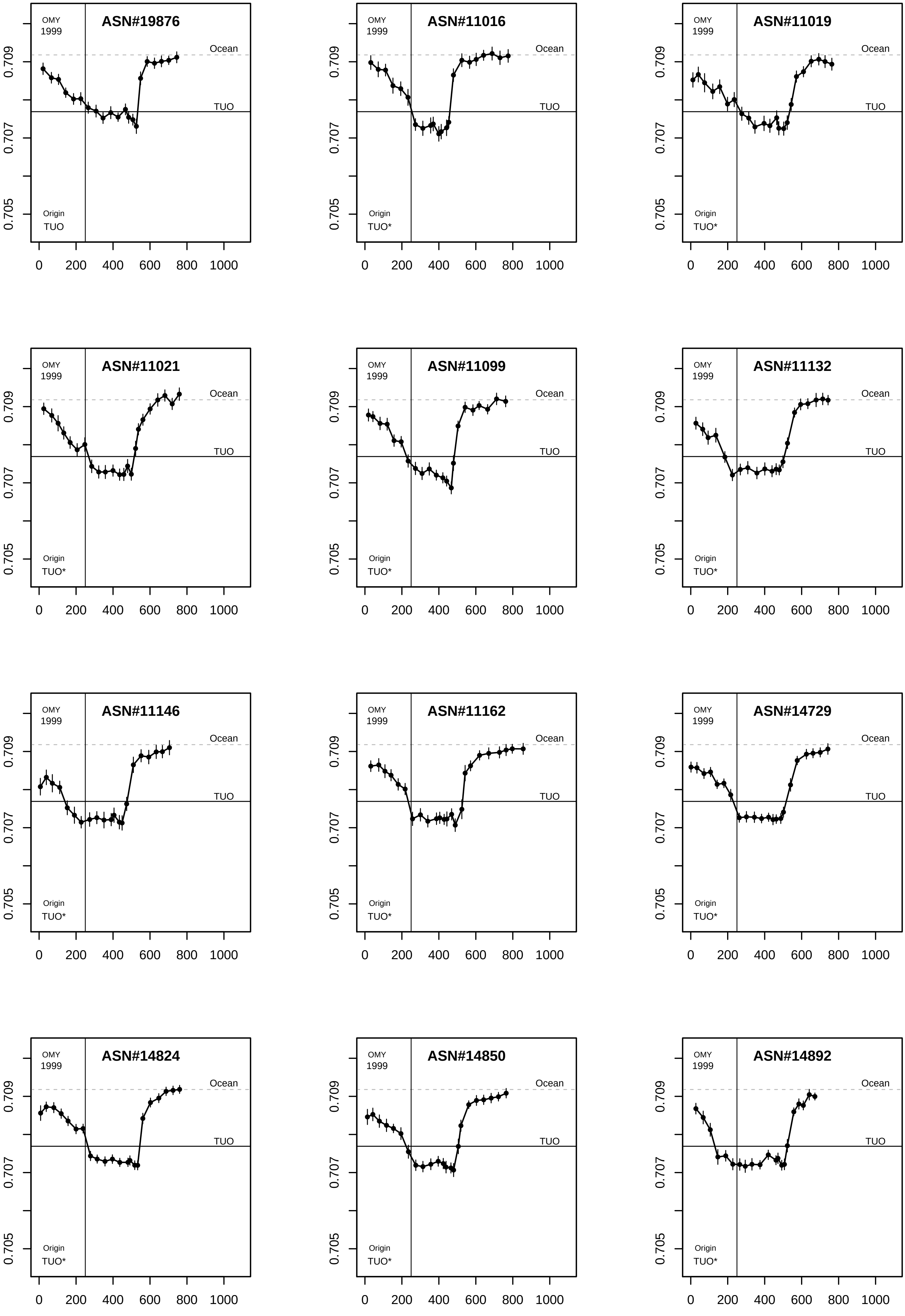
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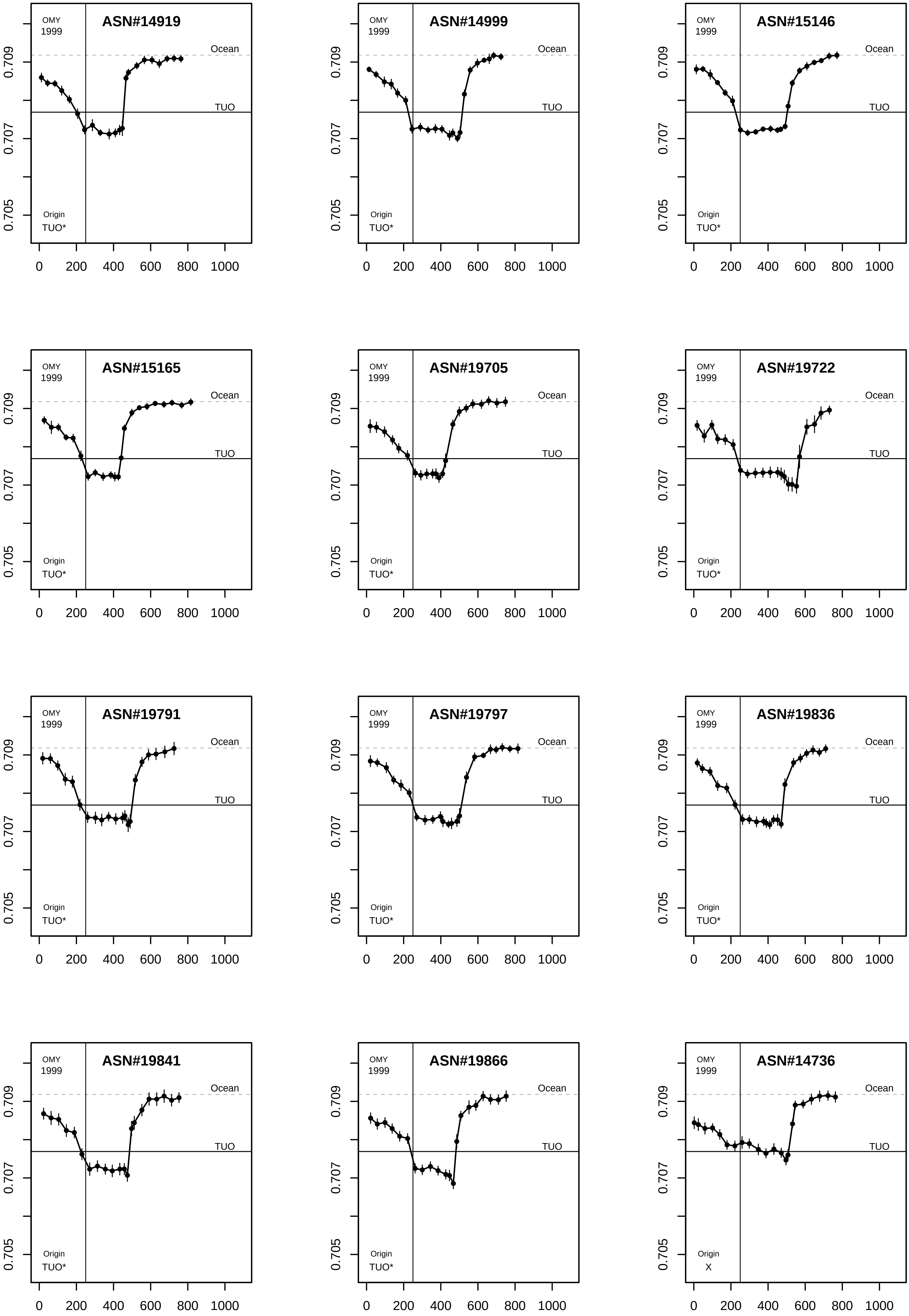

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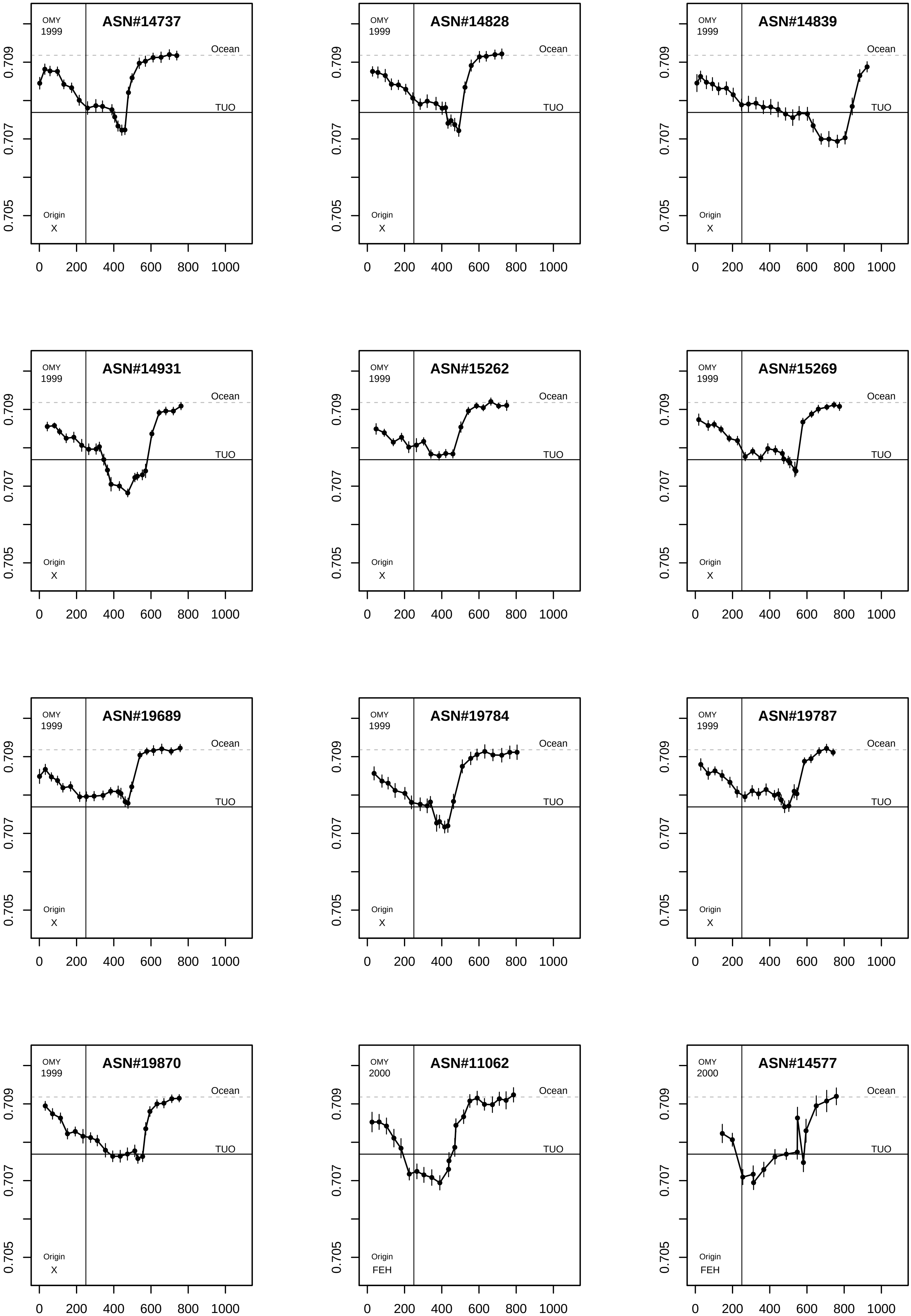
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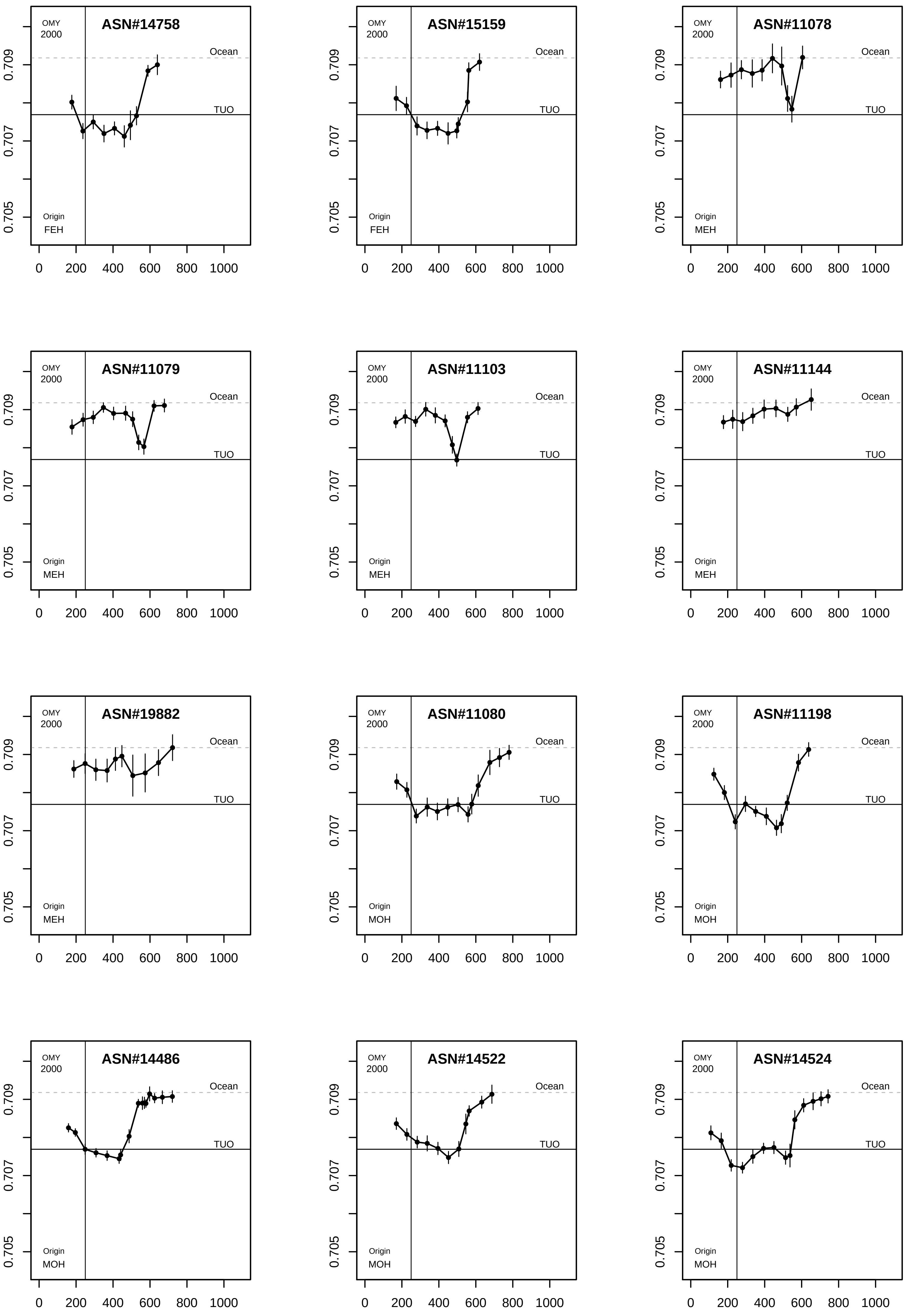
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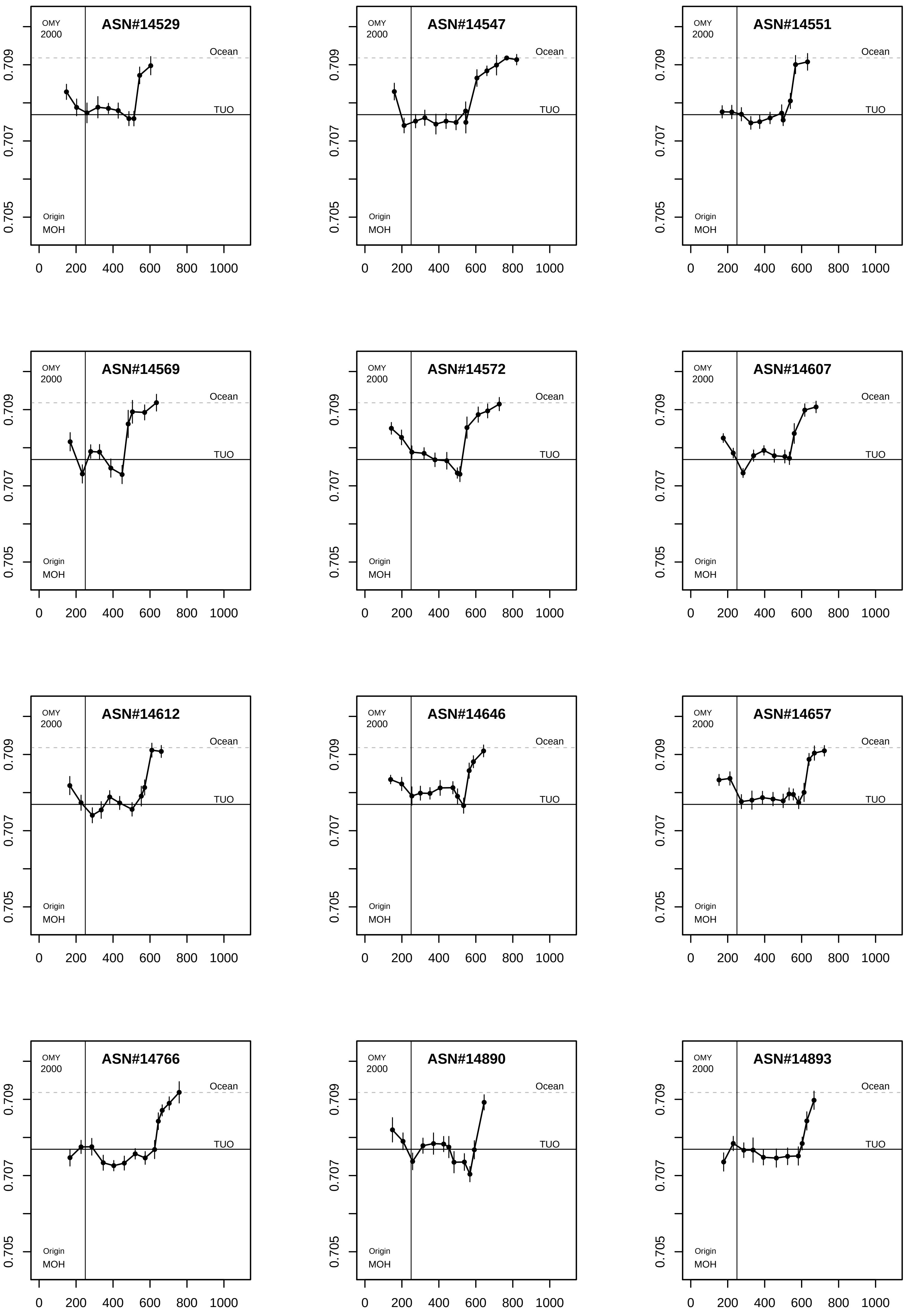
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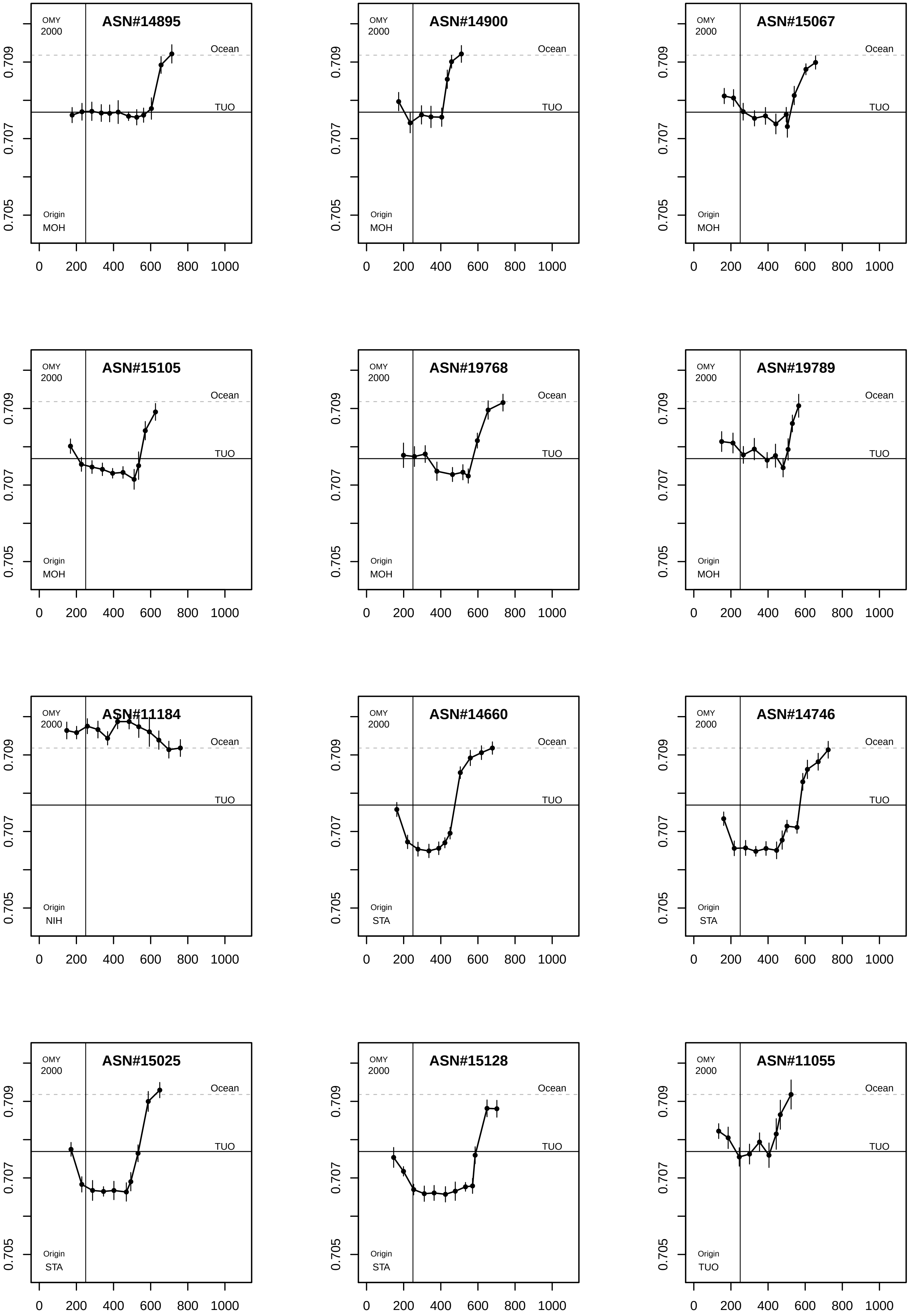
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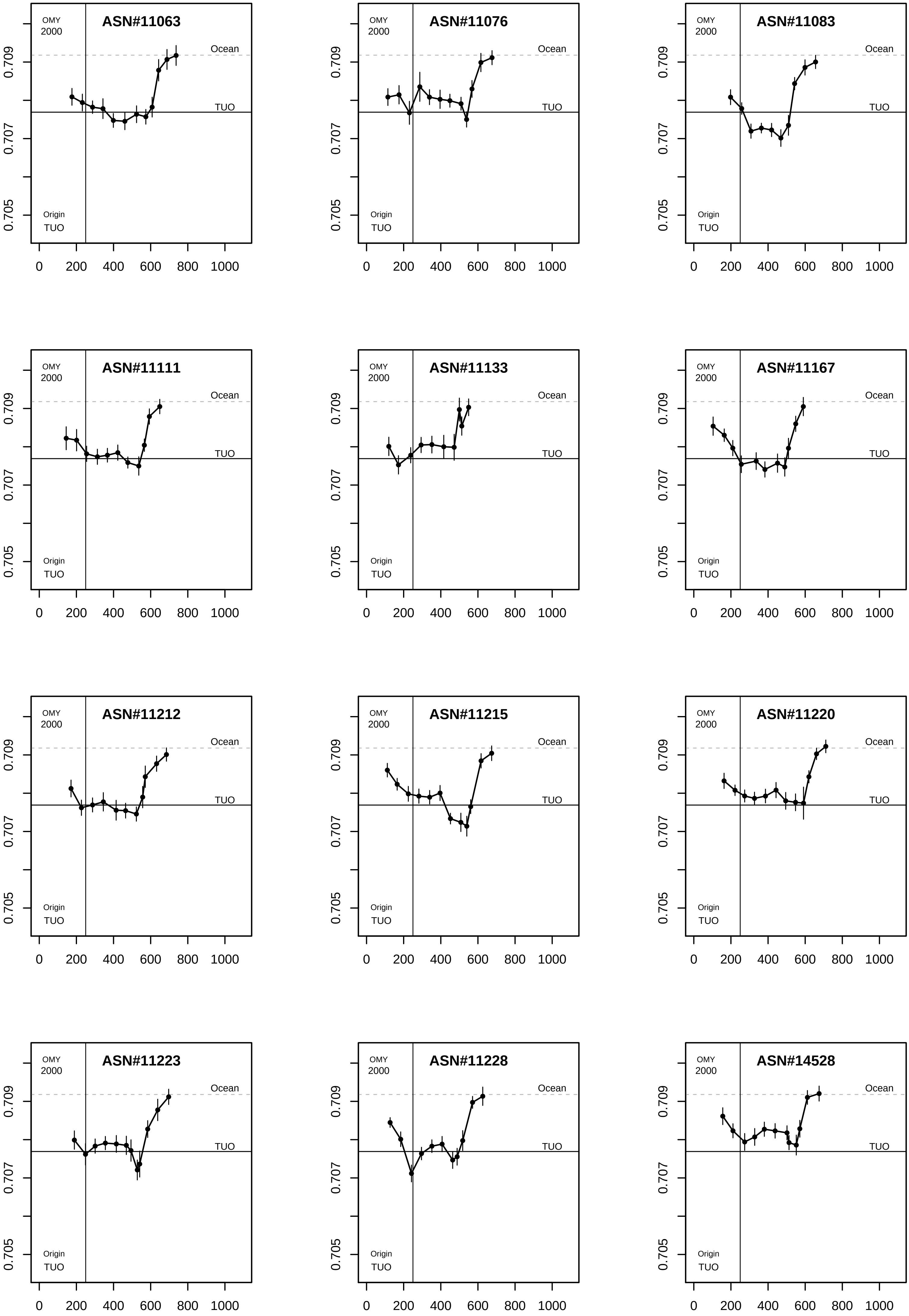
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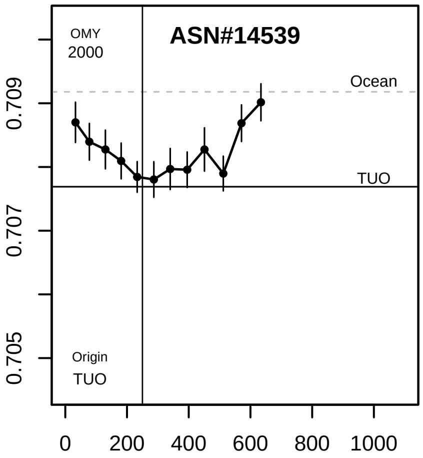
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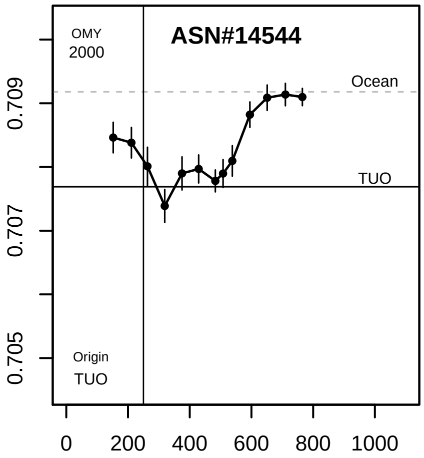
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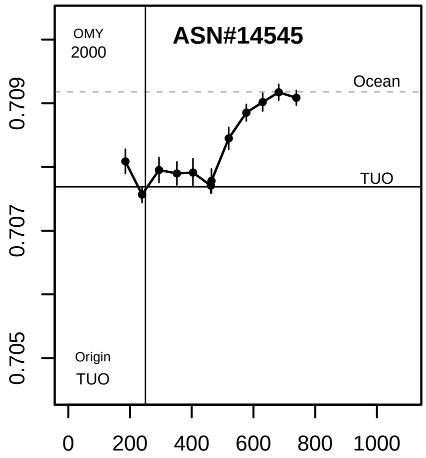
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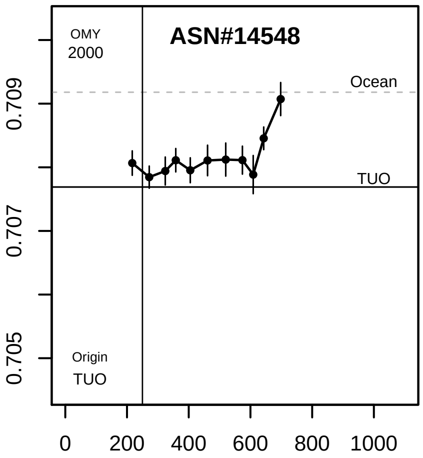
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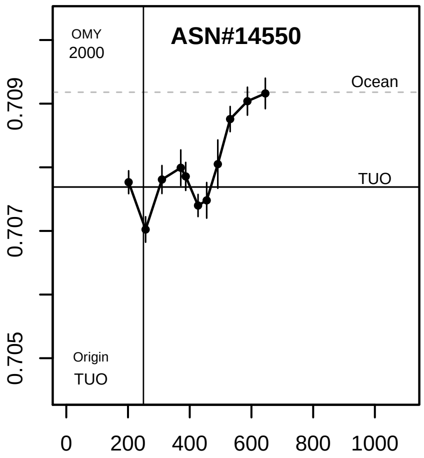
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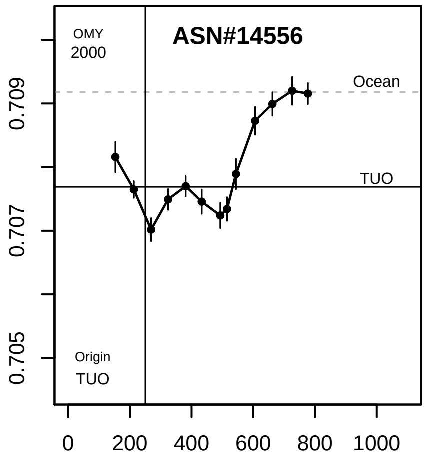
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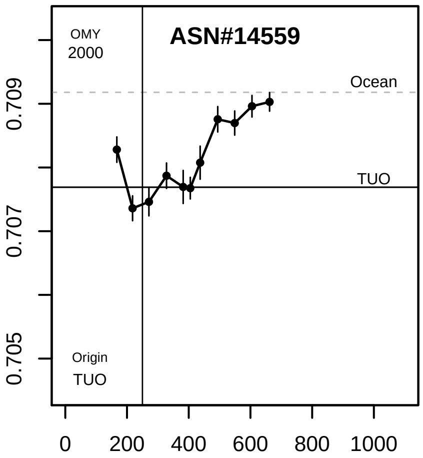
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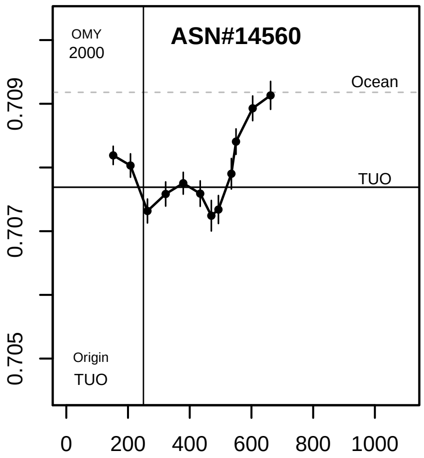
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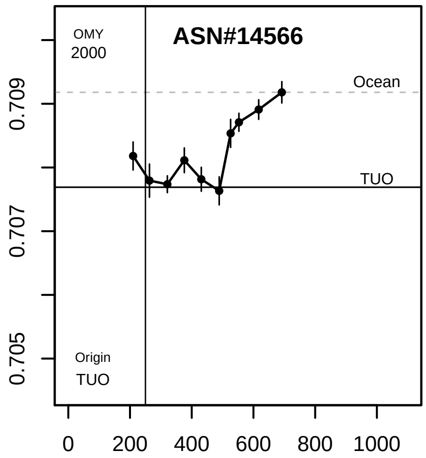
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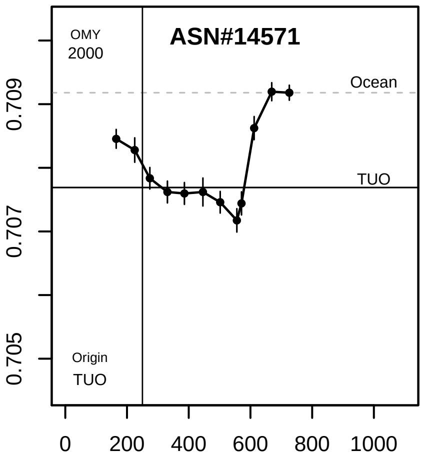
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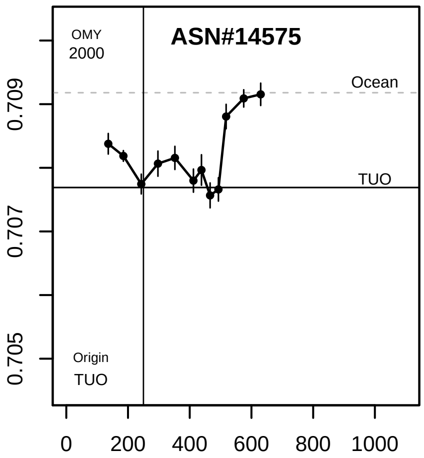
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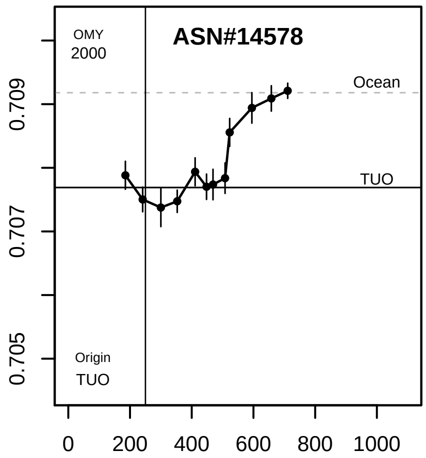
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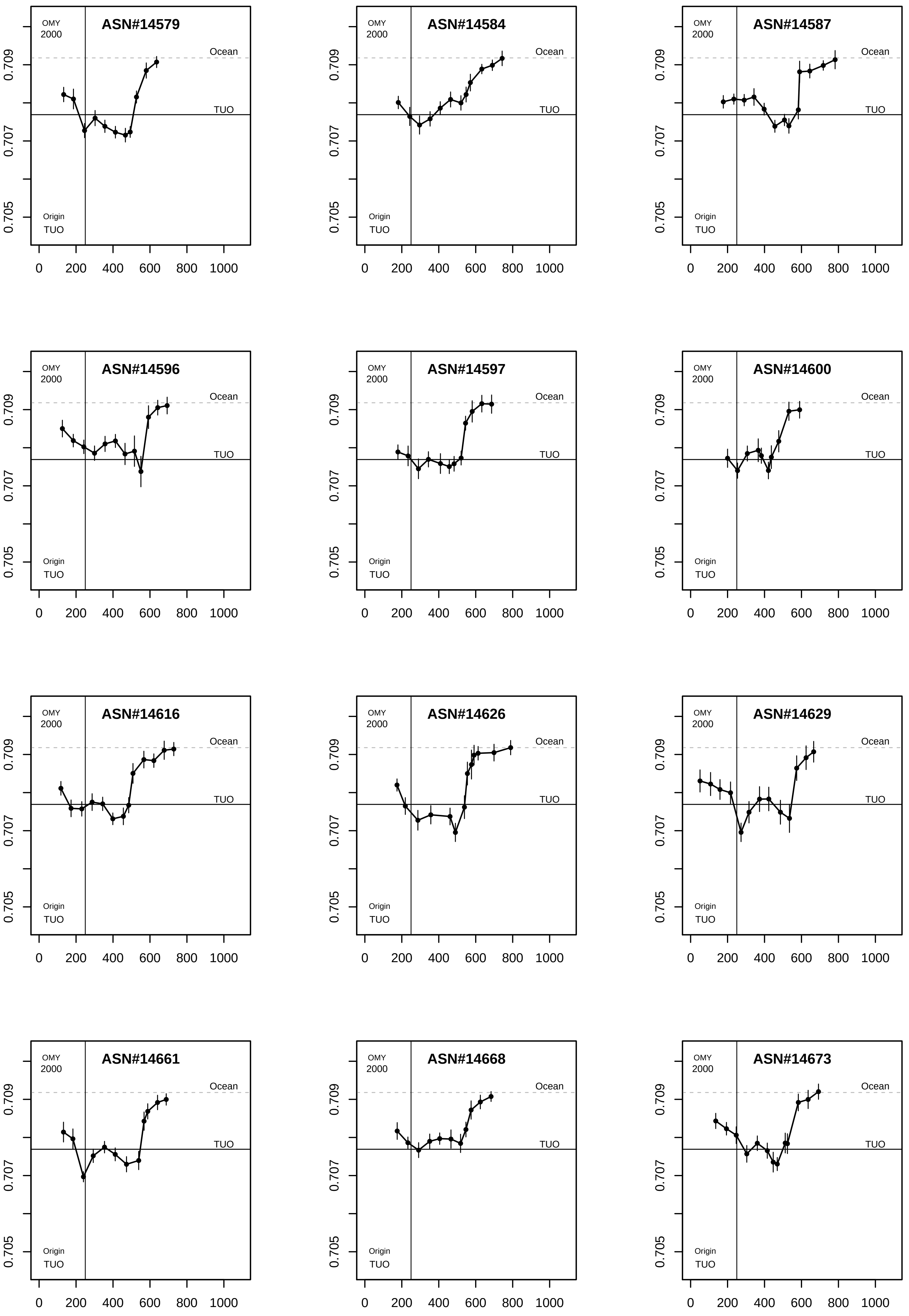
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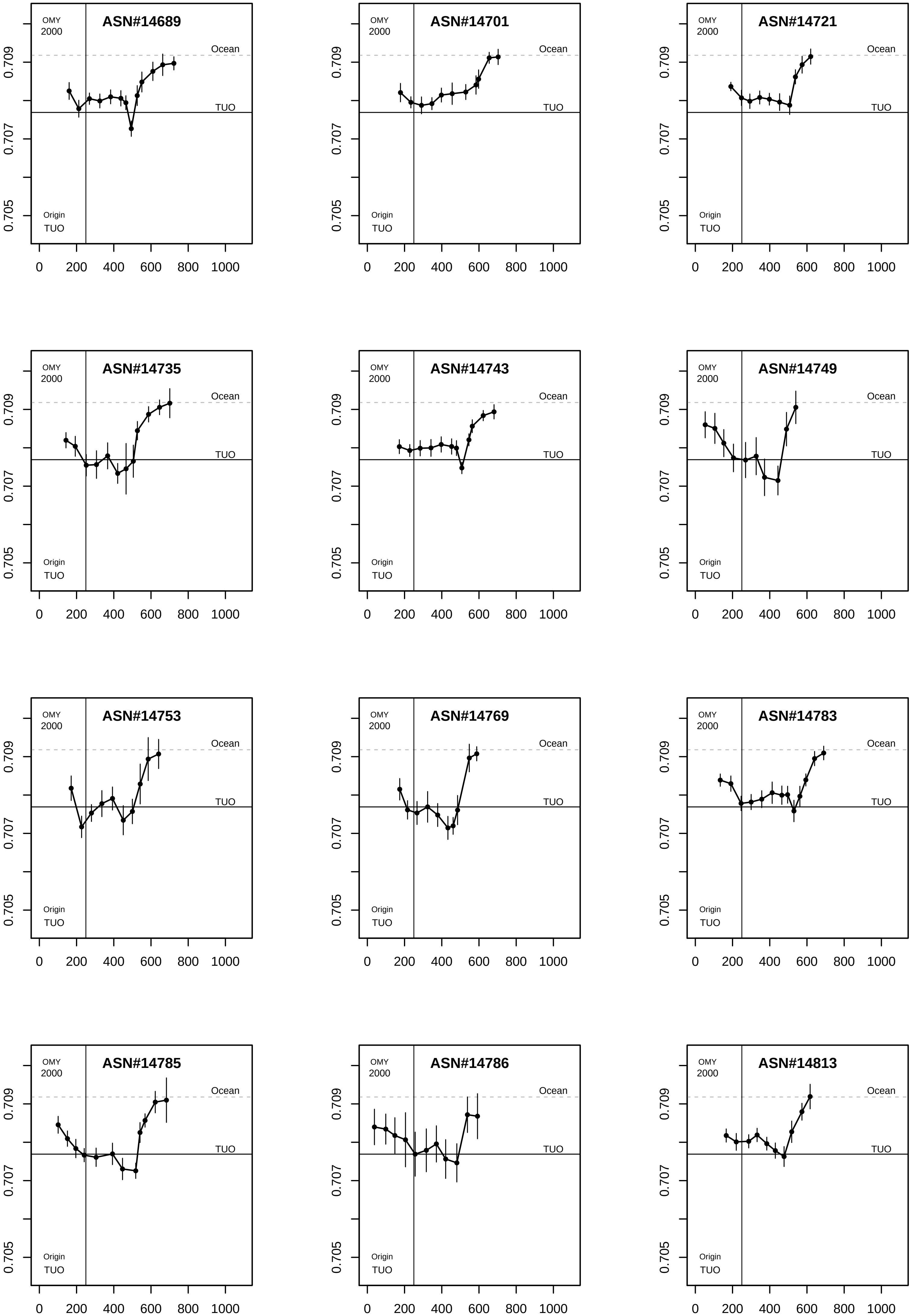
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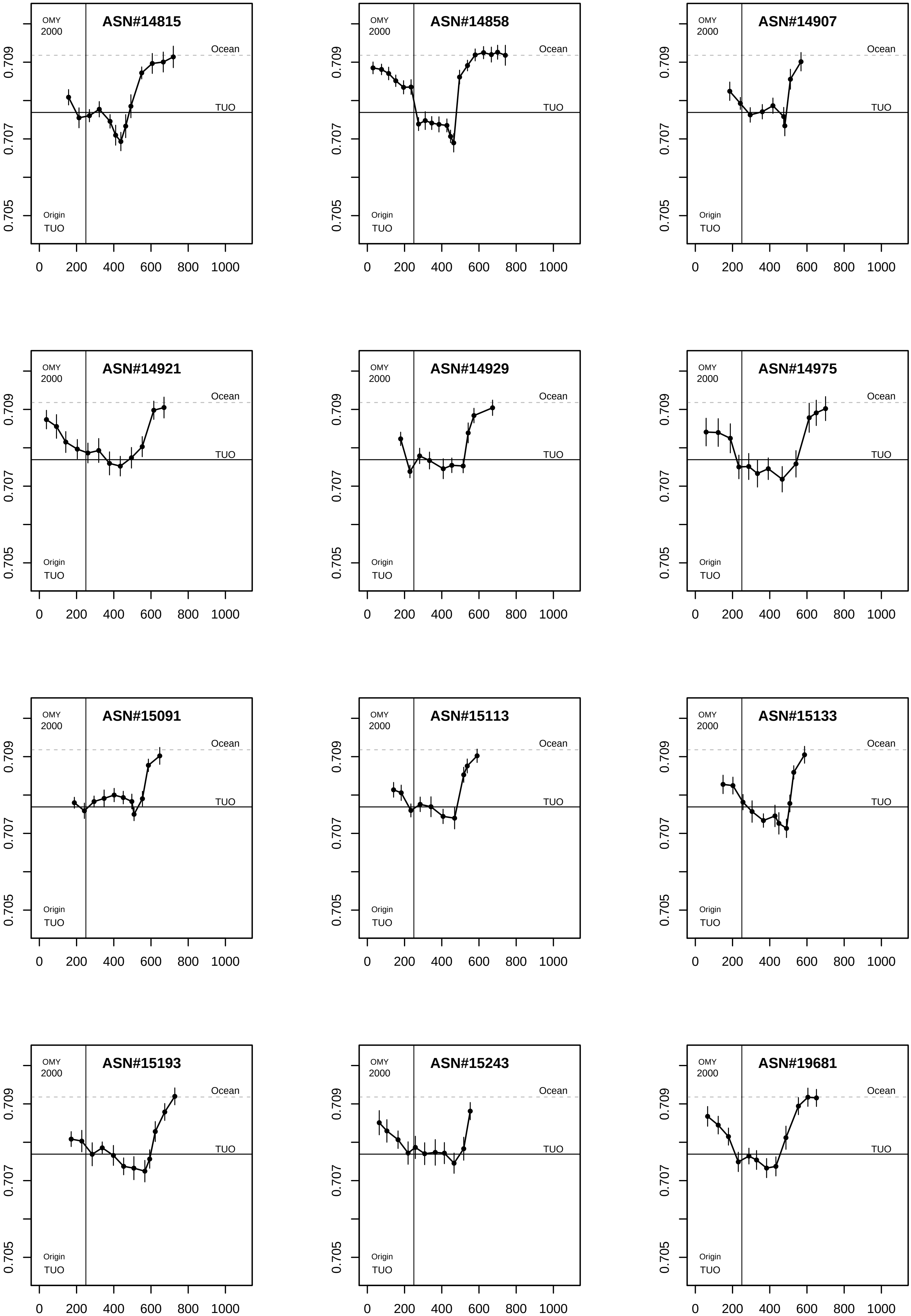
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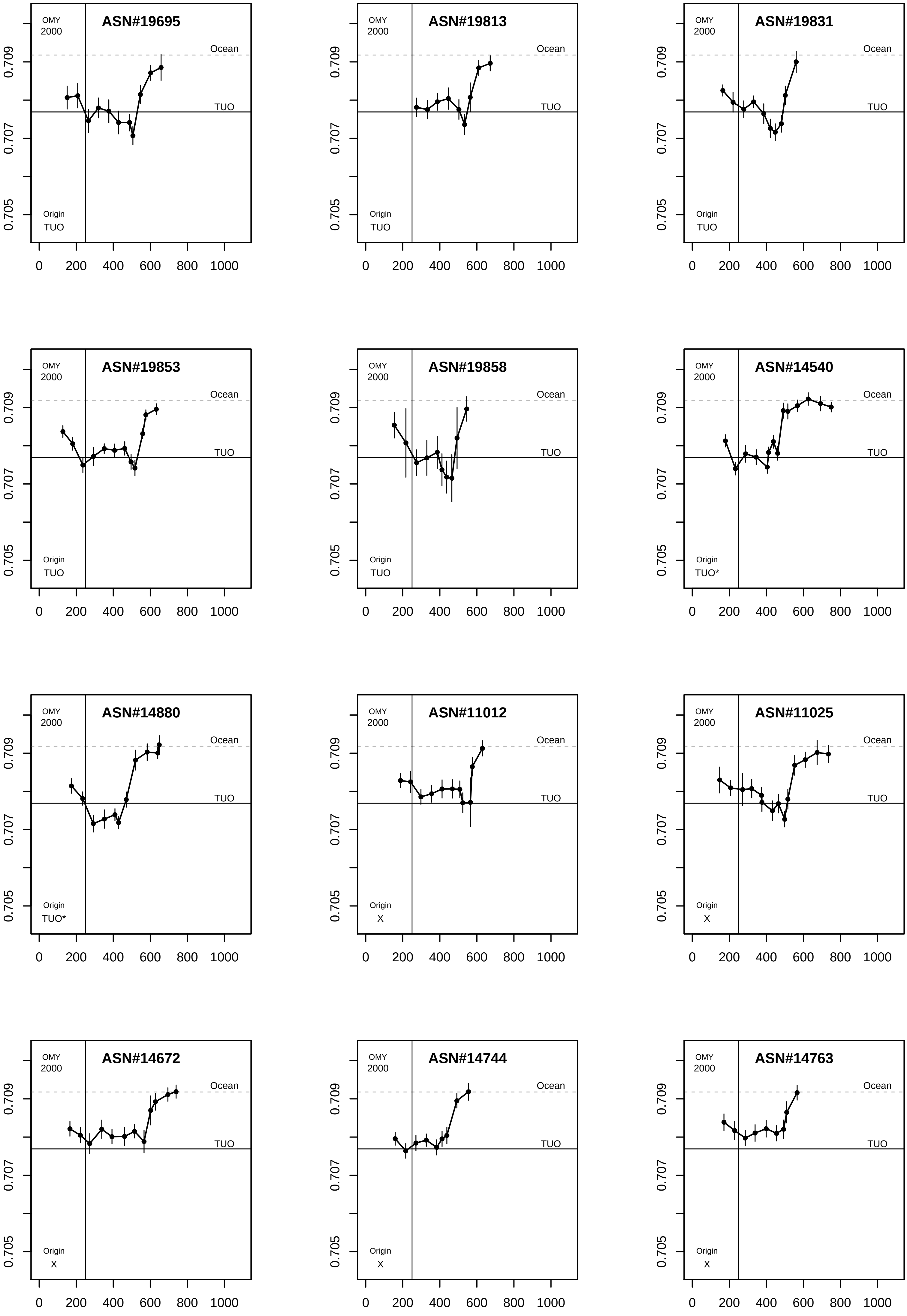

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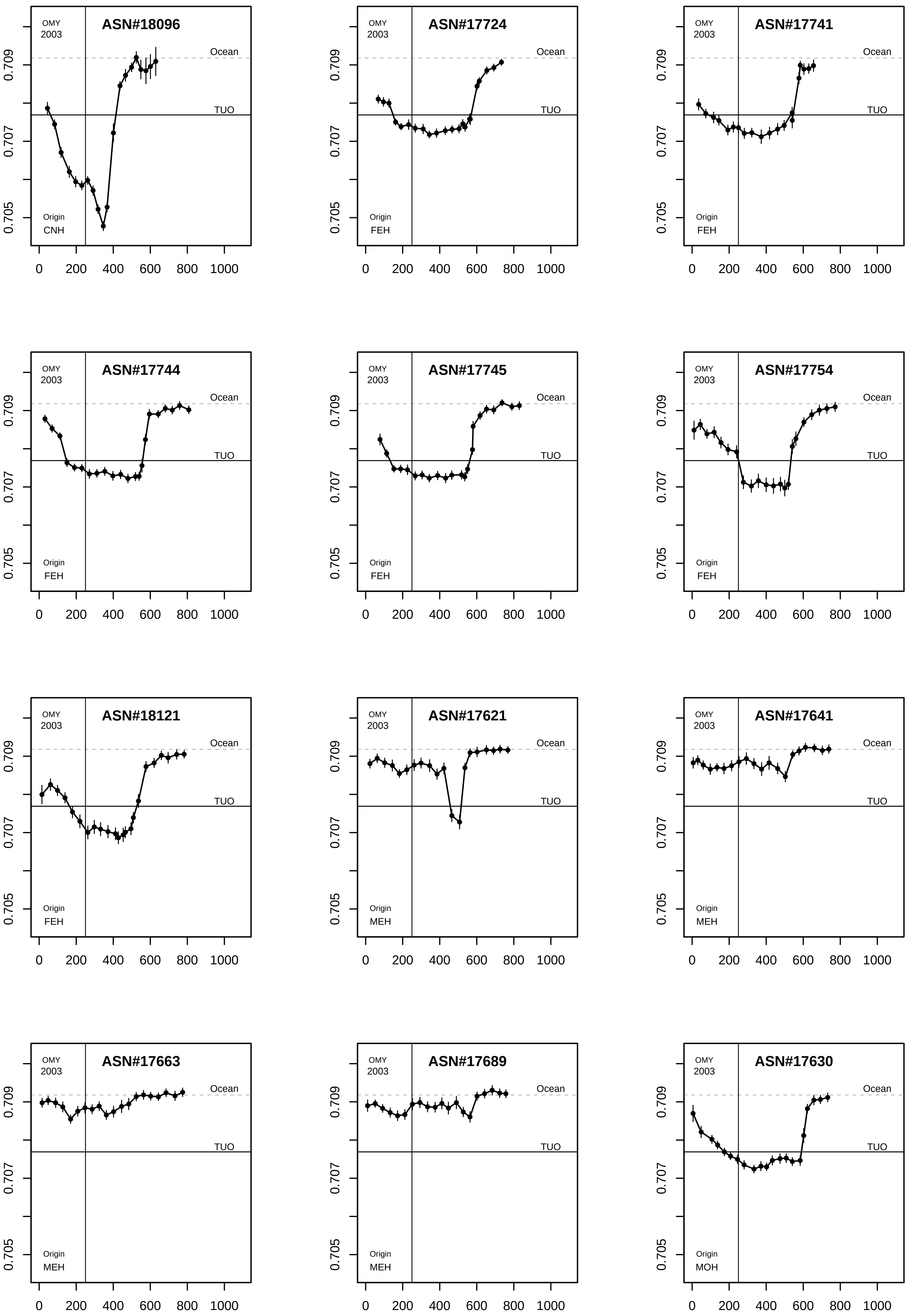

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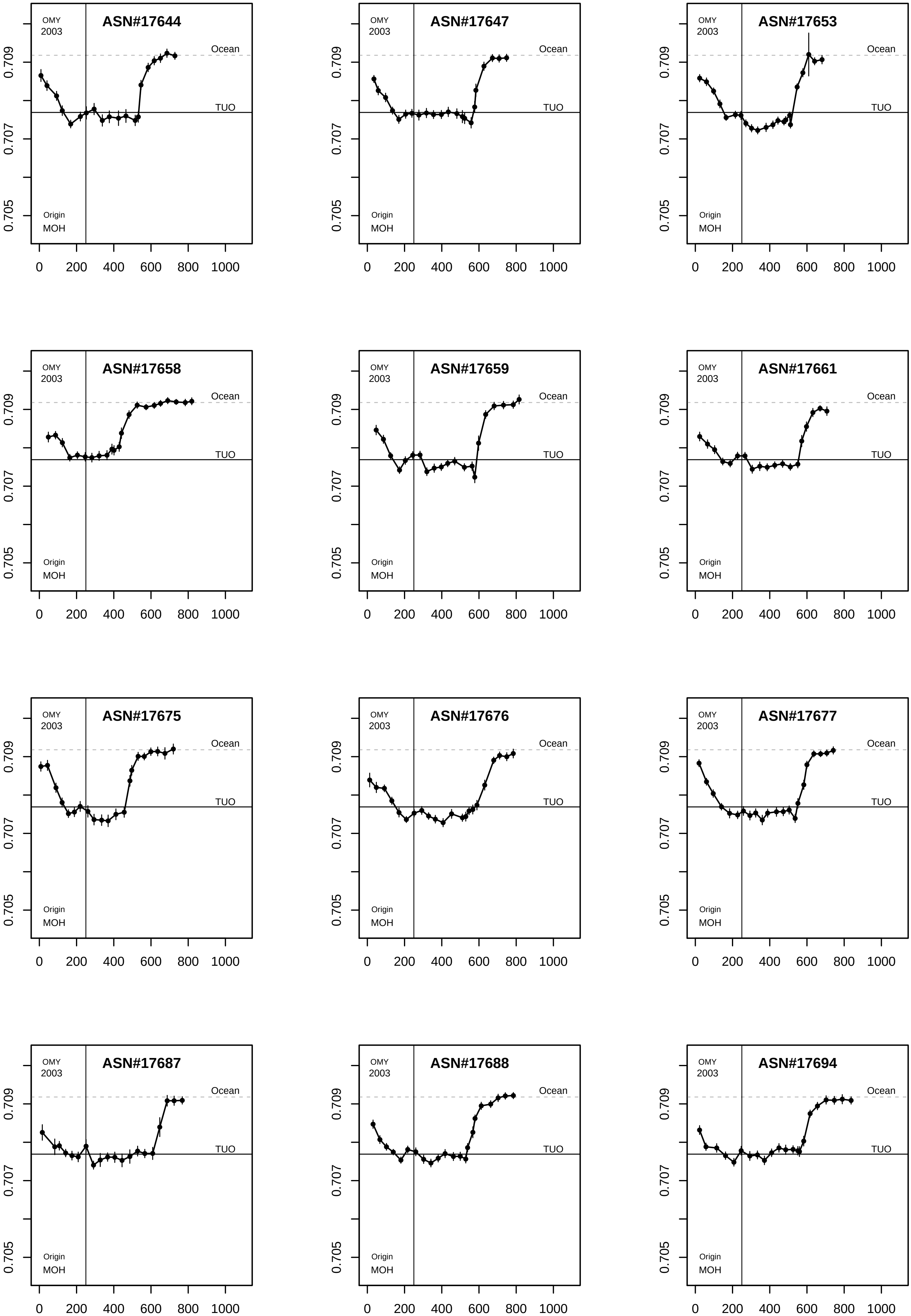
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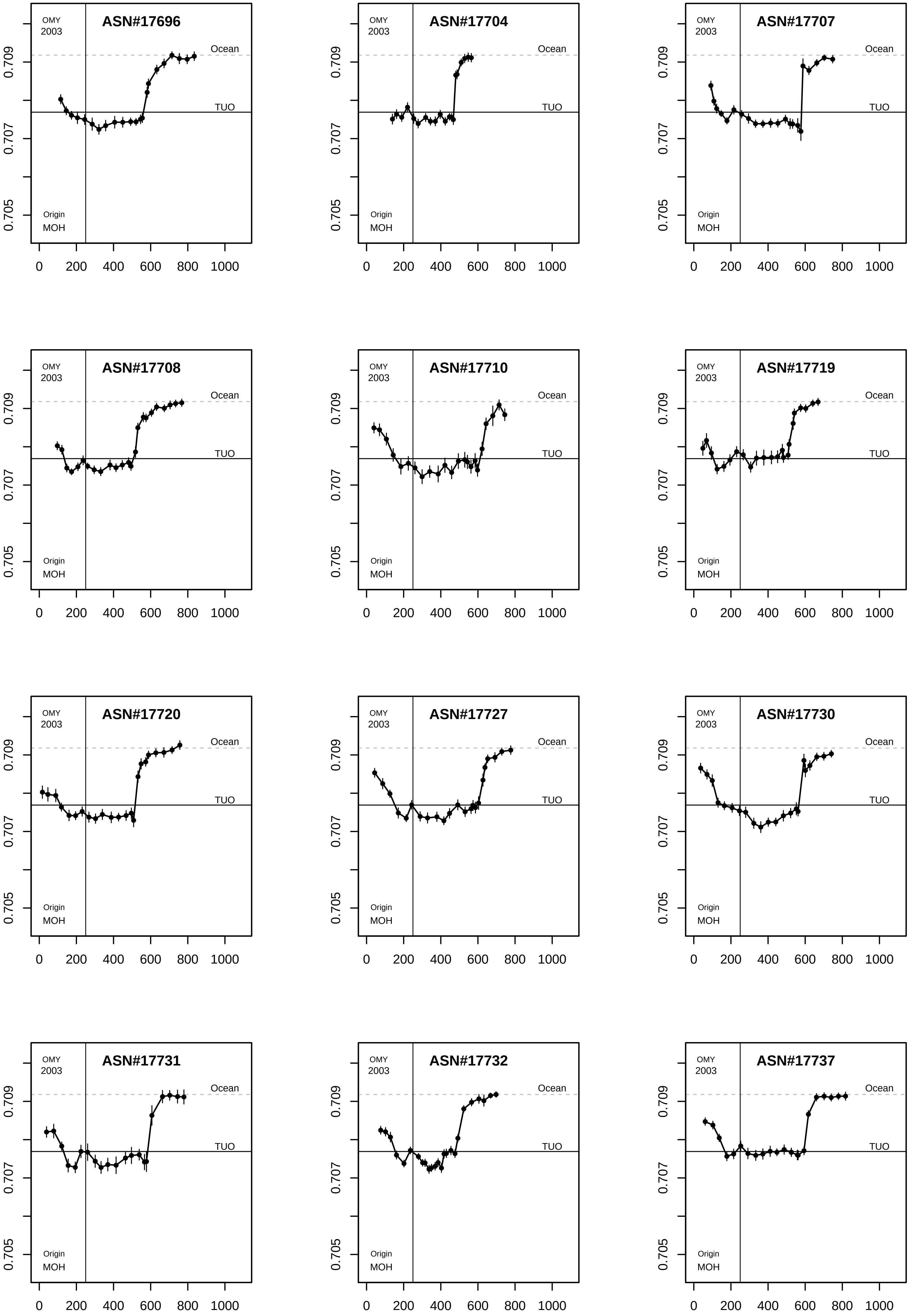
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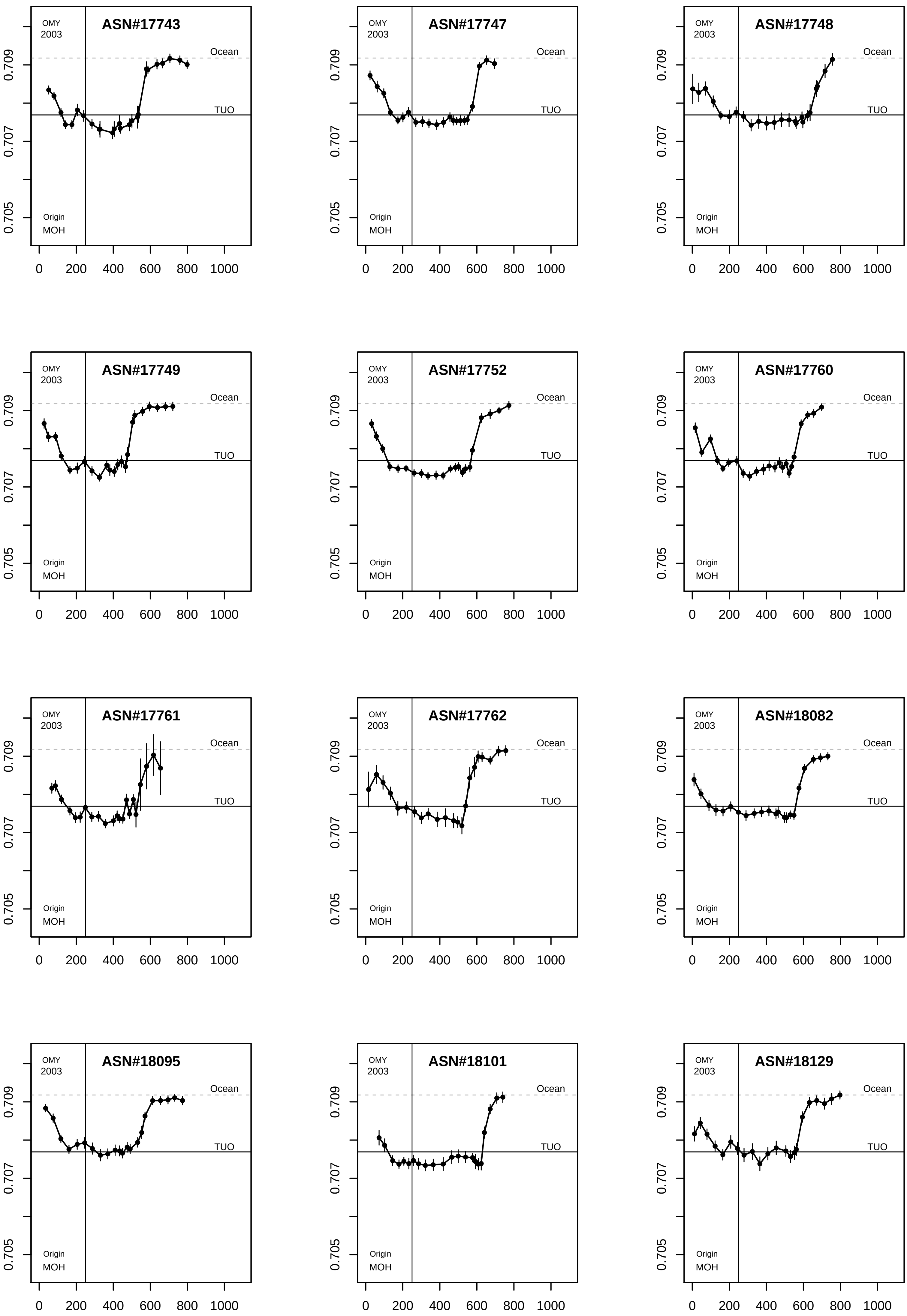

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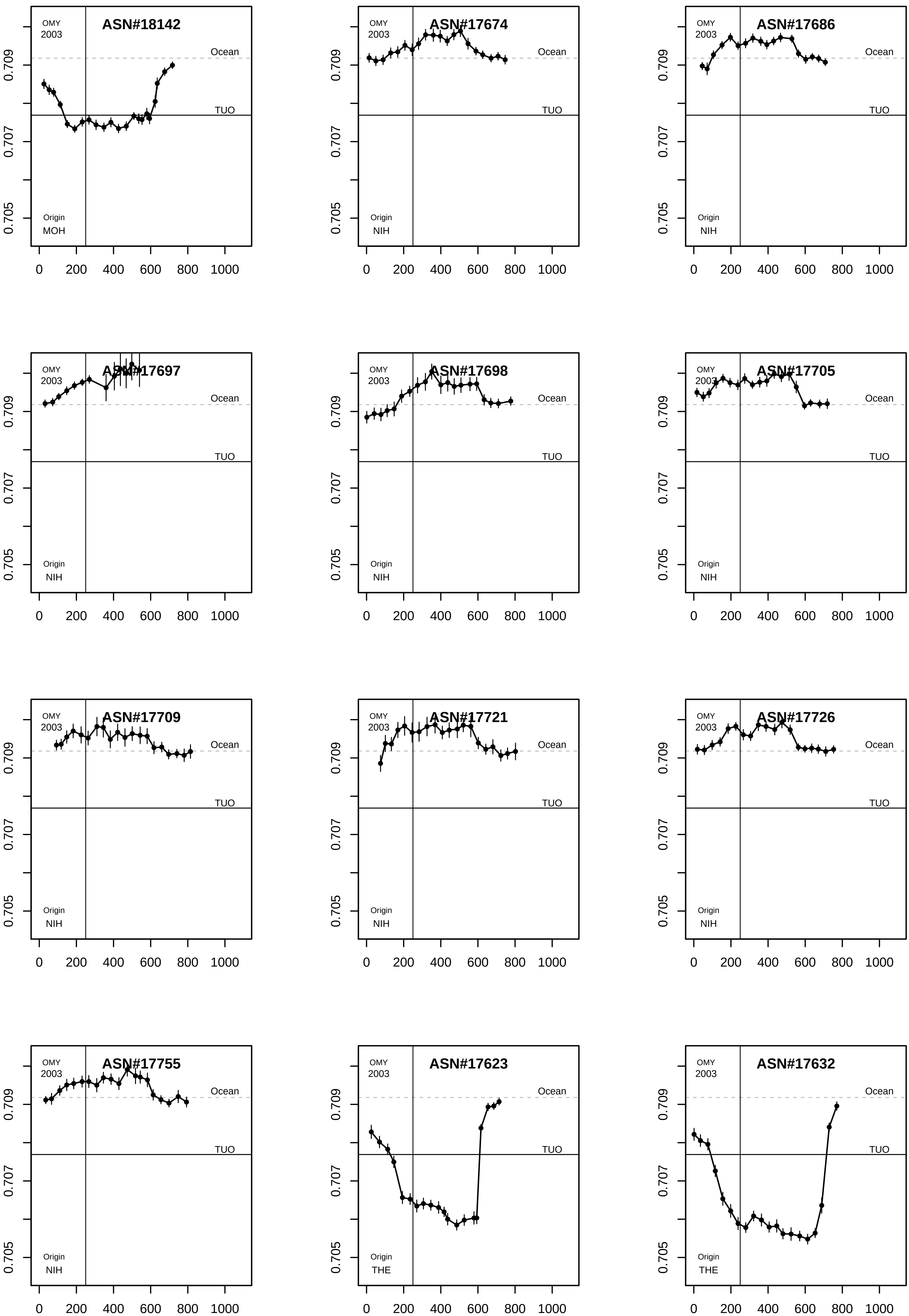
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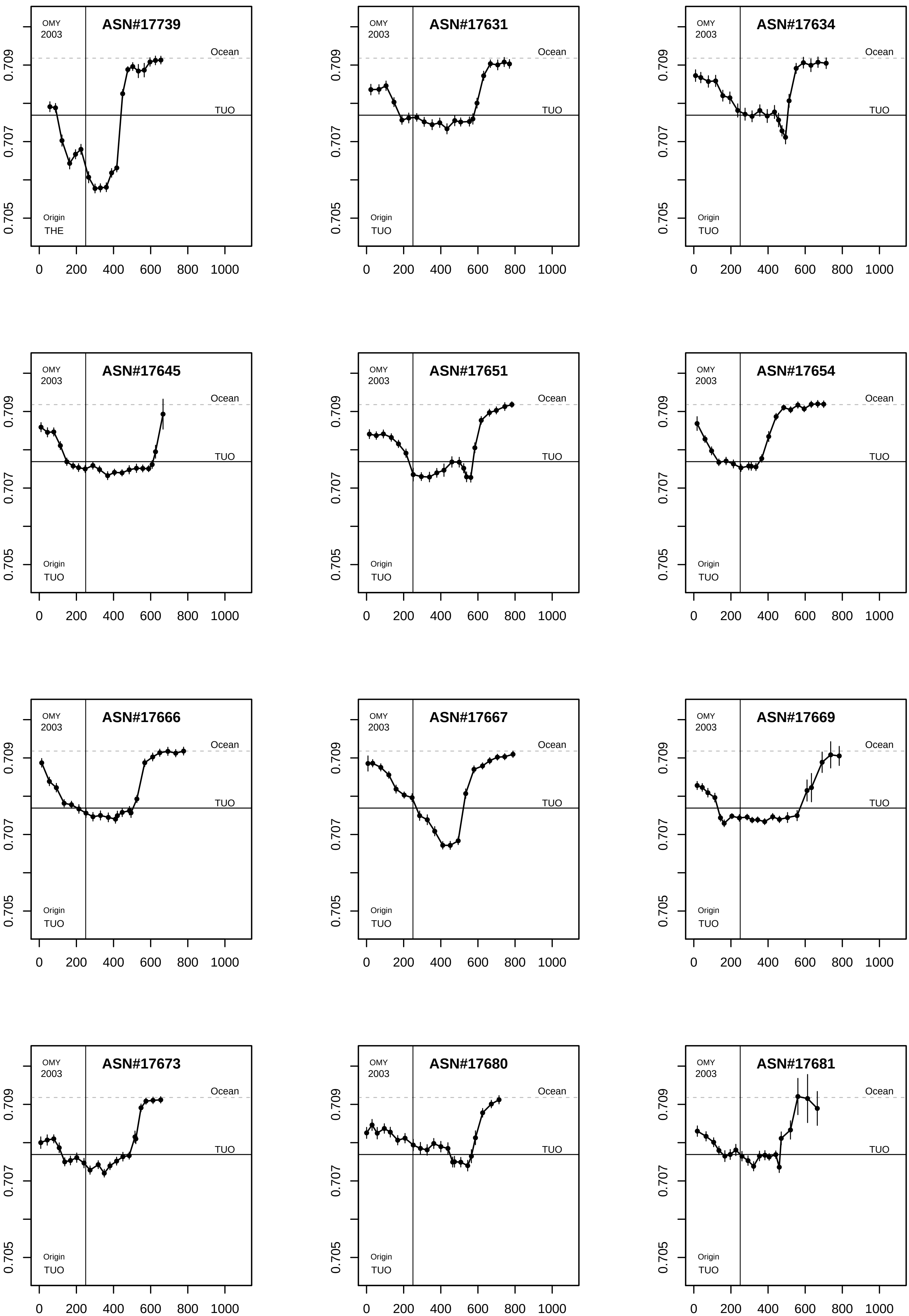
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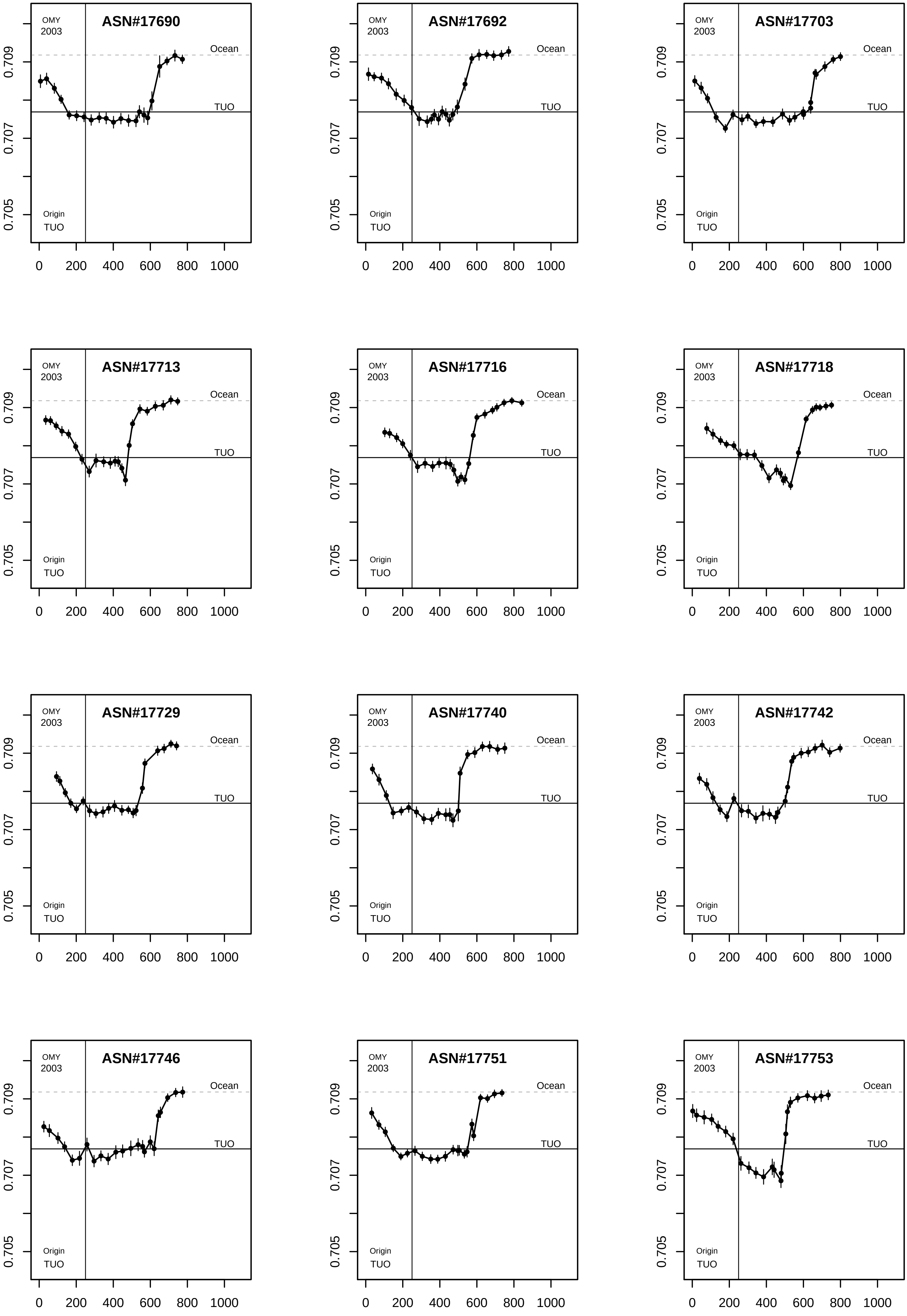
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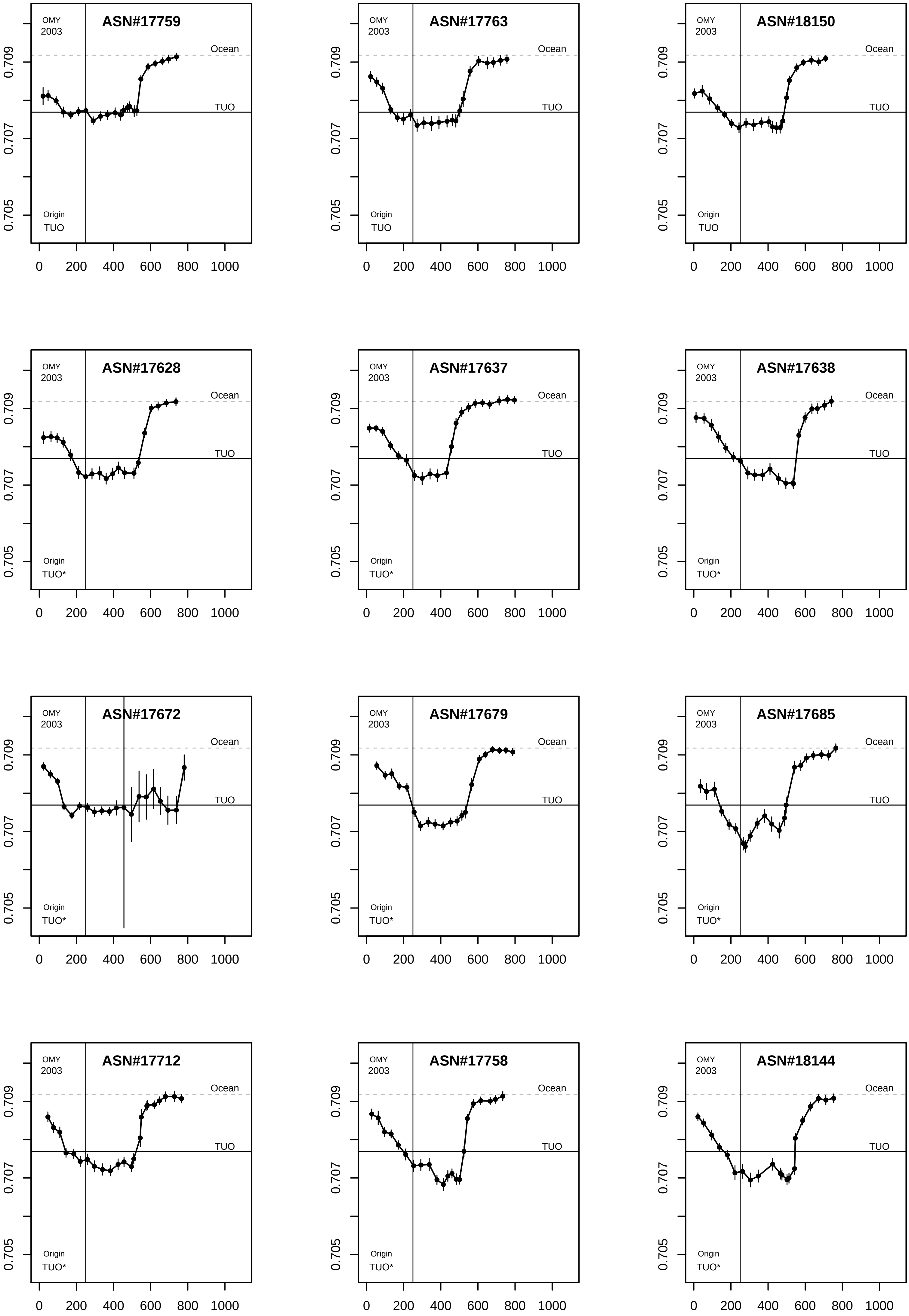
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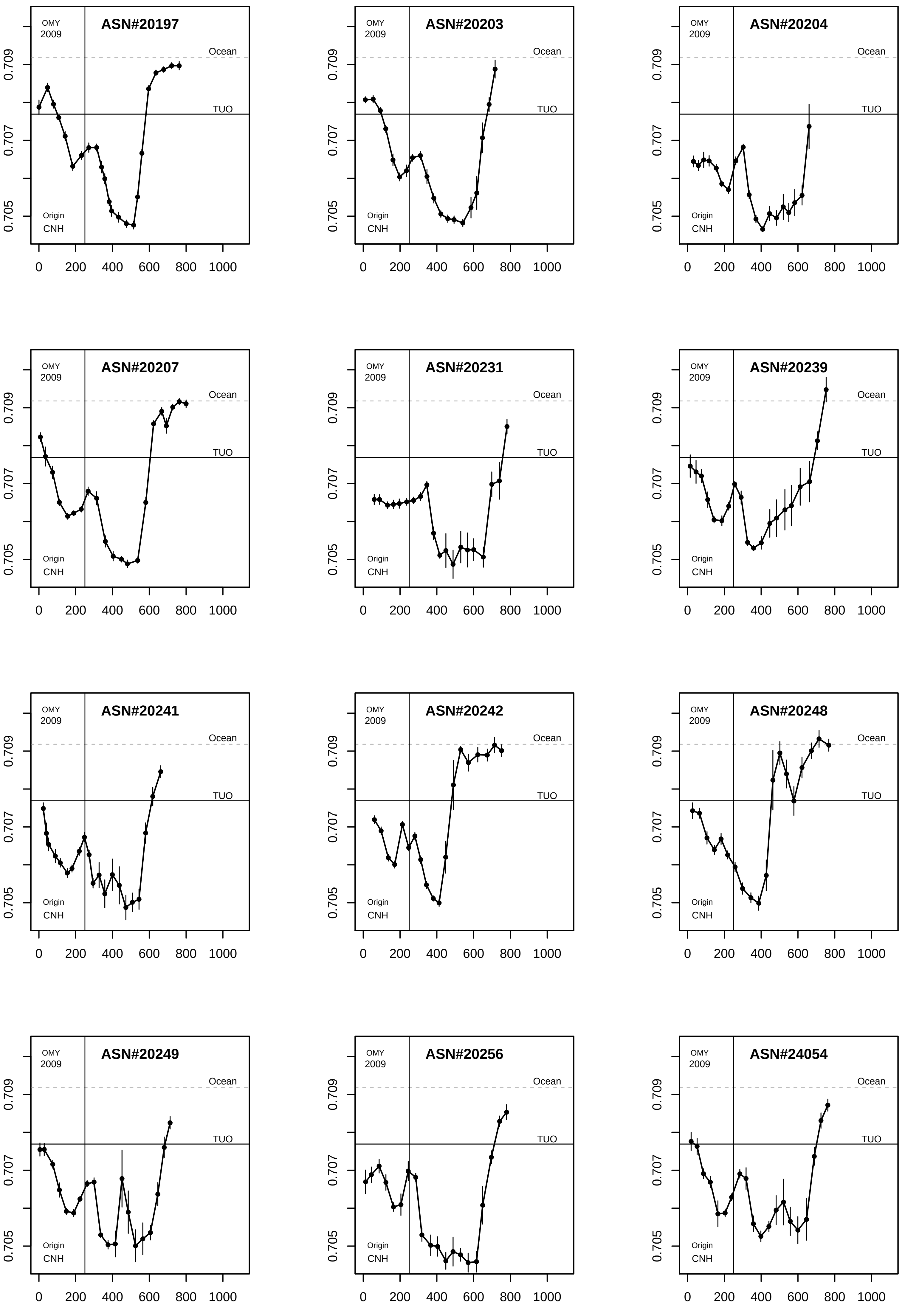
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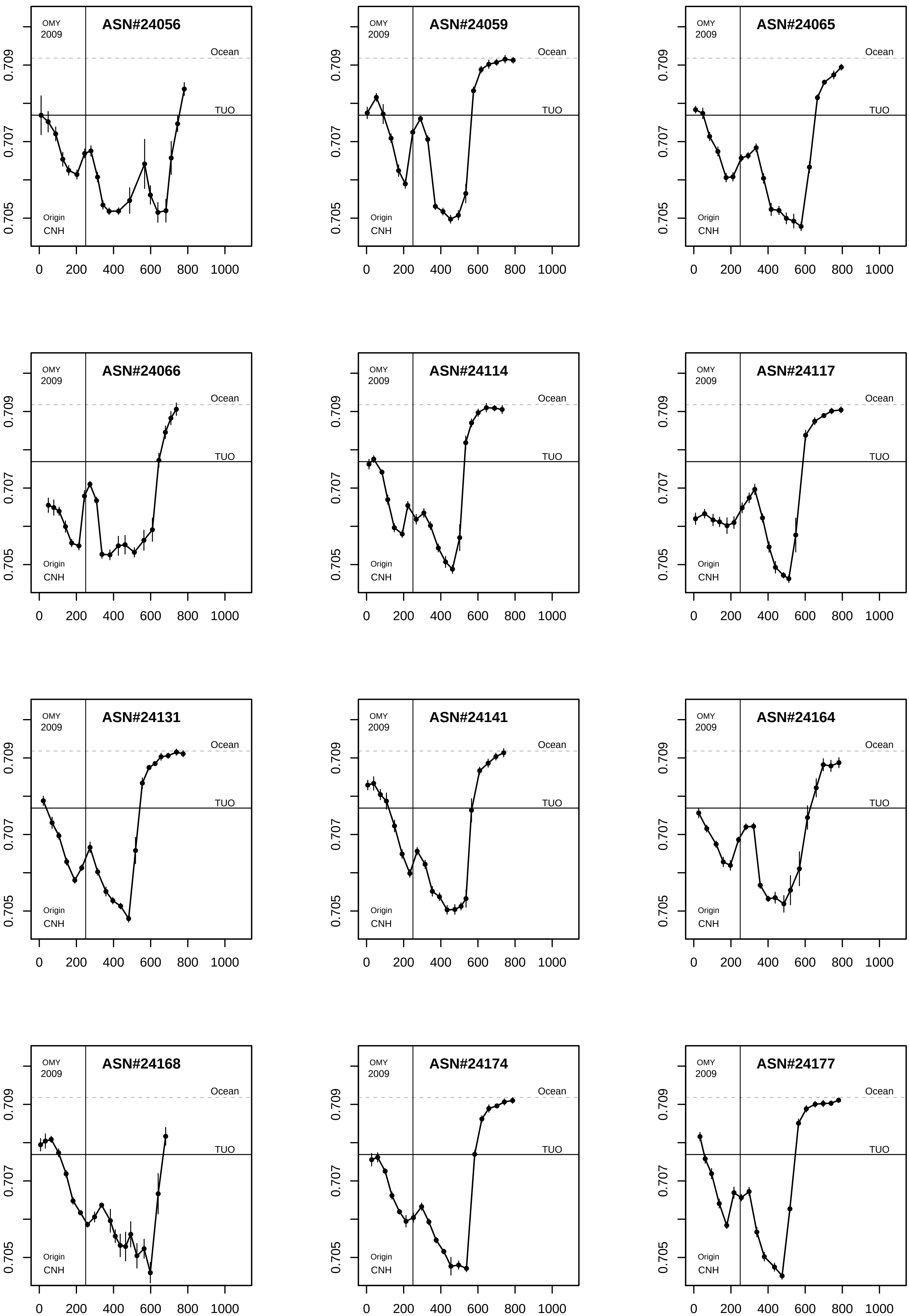
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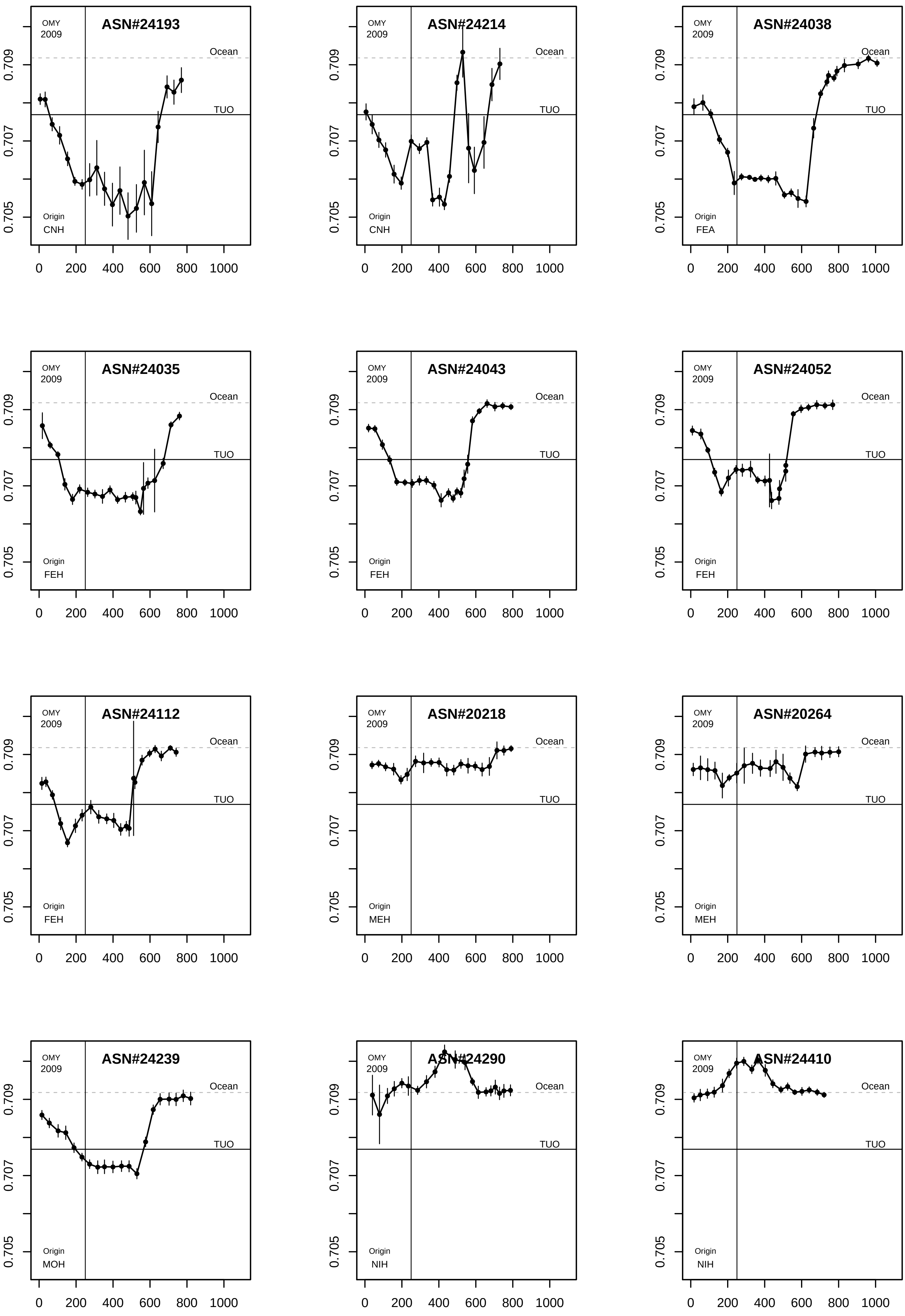
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