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Title: The Physiological Response of Larval Pacific lamprey to Chronic Heat Stress

Abstract approved:

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Scott A. Heppell

Pacific lamprey is an important cultural and ecological species to freshwater ecosystems of the Pacific Northwest. Lamprey often rear in low gradient portions of watersheds that have high exposure to climate warming, yet very little is known about their thermal physiology in comparison with other anadromous fishes such as Pacific salmon. Our goal was to characterize the response of larval Pacific lamprey to chronic heat stress to learn how changing stream temperatures could affect this life stage. Lamprey exposed in the laboratory to temperatures reflective of current summer water temperatures in the Willamette River, OR (20-25°C) did not suffer increased mortality. These animals did not feed substantially in captivity, inadvertently adding a starvation stressor to the experiment. Lamprey held at 22.5-25°C had standard metabolic rates approximately four times that of lamprey held at 13-25°C, which was associated with greater loss in both body mass and length. Additionally, the results of a differential gene expression (DE) analysis and functional enrichment analysis indicate pathways involved with starvation made up a significant proportion of the differentially expressed genes at elevated temperatures. These results suggest that

larval lamprey are able to resist multiple stressors for extended periods of time but likely at the cost of long term fitness and survival.

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The Physiological Response of Larval Pacific Lamprey to Chronic Heat Stress

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APPROVED:

Major Professor, representing Wildlife Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Patrick Carilli, Author

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DEDICATION

For Amelia…

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INTRODUCTION

Temperature has a prominent impact on the energy budgets of poikilotherms, and therefore a changing climate is likely to alter physiological processes in this suite of animals. Thermal performance curves peak at some thermal optimum before declining towards an upper lethal temperature, such as that seen in the salmonids (Schulte et al 2011, Gamperl et al 2002). These thermal performance curves have been used to set environmental policy to facilitate recovery of salmonid populations (Oregon Department of Environmental Quality 2020), such as the warm-water intolerant Chinook salmon (*Oncorhynchus tshawytscha*; Wurster et al 2005). However much less is known about temperature effects on non-teleost freshwater fishes.

Pacific lamprey (*Entosphenus tridentatus*) is a unique native fish intersecting both cultural and ecological aspects of the Pacific Northwest and is currently imperiled. They are culturally significant, traditionally being harvested for subsistence and cultural use by tribal communities including the Nez Perce Tribe, Confederated Tribes of the Umatilla Indian Reservation, Confederated Tribes of the Warm Springs Reservation of Oregon, Wanapum Tribe, Confederated Tribes of the Grand Ronde and Yakama Nation, among others. Ecologically, Pacific lamprey fill a number of roles. As larvae, their burrowing behavior improves habitat productivity by cycling nutrients and increasing sediment oxygenation. This is accomplished by the mechanical action of their burrowing exposing sequestered nutrients and allowing for greater water flow through the sediment analogous to earth worms in a garden (Shirakawa et al 2013). Both adult and larval lamprey are ecosystem integrators,

making both marine and sediment derived nutrients available to higher trophic levels including other fish, birds, and mammals (Close et al 2002). Pacific lamprey are declining region-wide (Clemens et al 2017), placing them on the sensitive species list in the state of Oregon (ODFW 2020).

Tributaries along the west coast of the United States including those of the Columbia River Basin provide nursery habitat for larval lamprey (Close et al 1995), and larvae can spend up to seven years buried in the sediment before metamorphosing and beginning the parasitic life stage (Beamish & Levings 1991). Adults spend a few years maturing at sea; they then migrate back upstream to spawn and die (Clemens et al 2013). Due to the cryptic and prolonged nature of the larval life stage, little is understood regarding how lamprey interact with potential environmental stressors.

The stress response in fish is described as a general reaction to a perceived threat (Schreck et al 2016); the physiological and biological pathways of the stress response can be divided into acute and chronic responses. Both acute and chronic stress have physiological and genetic indicators that can be measured including changes in body size, metabolism, and genetic markers that become activated in response to a stressor. The acute response is the immediate reaction of the organism to the perceived stressor, commonly referred to as "fight or flight". Close et al (2010) demonstrated that sea lamprey (*Petromyzon marinus*) use 11-deoxycortisol to signal the regulation of glucocorticoids and mineralocorticoids in response to acute stress. The chronic stress response of an organism is harder to define, as "chronic" is relative to the lifespan of an organism; it can take place over the course of days or months depending on the organism. For this study, we consider chronic stress for larval

lamprey as a prolonged exposure to a sub-lethal stressor lasting longer than a month. There is currently limited understanding of the impacts that chronic heat stress might have on Pacific lamprey larvae, and less still is understood regarding the interplay of multiple stressors acting on their physiology. Both adults and eggs of lampreys have an estimated lethal thermal threshold around 27°C (Clemens et al 2016) but temperature maxima and optima are inexorably tied to food availability and energy stores which in turn dictate aerobic scope.

Our goal was to quantify the physiological response of larval Pacific lamprey to chronic (90d) heat stress. Specifically, we sought to: (1) determine how the metabolism of Pacific lamprey larva is altered when exposed to elevated temperature, and (2) determine the chronic effects of temperature on the body size of larval lamprey. This includes measuring metabolic rate, growth rate and genetic indicators of stress through a differential expression (DE) approach. Specifically, we expected a significant portion of differentially expressed genes to be associated with the heat shock response. We hypothesize that fish exposed to a non-lethal elevated temperature will demonstrate an accelerated growth rate compared to control animals until a lethal temperature is reached.

MATERIALS & METHODES

Collection & Husbandry: Animals were collected from the Mary's River (a tributary of the Willamette River) near Philomath, OR (USA). The Mary's River has a rain-dominated hydrology and exhibits an annual maximum temperature of ~24°C (Pearcy 1999). Animals were captured in the morning between 7:30 am and 12 pm on three different days in July of 2018. We used an AbP-2 backpack electrofisher (Engineering Technical Services, Madison, WI, USA) designed for larval lamprey collection, using low current to probe the sediment and disturb larval lamprey from their burrows as per Schultz et al (2014). We captured 243 animals initially placed them in a bucket of river water with ice packs, then transferred to a second bucket filled with river water, tricaine methane sulfonate (MS-222,0.050g/L), and bicarbonate buffer (0.125g/L) to render fish unconscious for handling and measurement. Fish remained in the MS-222 bucket until the animals lost equilibrium and stopped responding to stimuli.

Each lamprey was placed on a measuring board for a positive identification, using the techniques developed by Goodman et al (2009). Pacific lamprey were further scrutinized for signs of metamorphosis, illness, or damage. Animals > 150mm, with developing or developed eyes, or that had developed teeth indicative of transformers and juveniles, respectively (Clemens 2019) were not retained. Lamprey that were retained (243) for the study were placed in a cooler with river water, ice packs to prevent overheating during transport, and an air pump to recover from anesthesia. Animals that did not meet the specifications of the study were placed in a recovery bucket of river water before being released back into their habitat. Collected

animals were then moved to the Oregon State University Aquatic Animals Health Laboratory (AAHL), in Corvallis, OR (USA).

Lamprey husbandry techniques were adapted from the work of Lampman et al (2016). Larval lamprey are filter feeders, using their gill basket to capture suspended organic partials and thus the holding facility was designed to facilitate this (Mallatt 1981). At the AAHL, larvae were placed in cylindrical 200ml plastic containers filled with commercially available playground sand and capped with a mesh lid. The containers were then placed in a 100L tank at 13°C to begin the acclimation process. The diet of the animals consists of Fleischmann's activated yeast and San Francisco Bay Brand brine shrimp slurry. Feeding took place three times a week for 30 minutes during which time the water flow was turned off allowing the food to settle so the lamprey could feed. Each lamprey received ~0.5g of food per week. Tank temperatures were monitored with an electronic thermometer (+/- 0.1°C) during feeding. After 30 minutes, water flow was reactivated. This feeding regimen was selected to maintain tank temperatures as initial testing showed temperature fluctuations in a tank if water flow was off for longer than 30min. Tanks were cleaned once a week using a siphon. During this time, lamprey were visually checked for signs of illness (such as fin rot) or morbidity. Animals that were ill were placed in a lethal solution of buffered MS-222 (0.4g/L with 1.0g/L bicarbonate buffer) followed by the severance of the spine for euthanasia. A total of 15 lamprey experienced morbidity during the 30d acclimation period leaving 228 to conduct the study with.

Treatments: To assess the effects that long-term temperature stress has on the physiology of larval Pacific lamprey, animals were exposed to elevated temperatures

during a 90-day period. A total of 228 animals were split between one of five treatments 13°C (control), 15°C, 20°C, 22.5°C, and 25.0°C). Due to logistical constraints, only three temperatures could be run concurrently (Appendix Table 2). An initial 144 lamprey were used to test the first three temperatures (13°C, 15°C, 20°C) while the remaining 96 were held at 13°C until the experiment was repeated with the reserved animals at the higher temperatures plus control (13°C, 22.5°C & 25°C). Each animal was randomly assigned to one of nine tanks holding 9-17 lampreys per tank, with each lamprey isolated in its original 200ml container. Each temperature treatment had three replicate tanks for a total of ~48 animals per treatment. The water temperature in each tank was increased by 1°C per 24h until the desired temperature was reached. Treatments with a higher final temperature were started earlier so that all treatments simultaneously reached their final target temperature.

Growth Sampling: Prior to the start of the temperature exposure, body mass and length data were collected for each individual. Animals were separated from the substrate by gently straining the sand through an aquarium net and then anesthetized in a container of buffered MS-222 (0.05g/l) equipped with an aeration pump, and ice packs to prevent overheating. Once equilibrium was lost and movement ceased, each larva was placed on a measuring board and length was measured to the nearest mm. To record body mass, each animal was first placed on a damp sponge to remove excess water and then weighed to the nearest 0.001g on an analytical balance. The lamprey was then placed back in its 200ml container of sediment and given time to recover in a separate bucket before being placed back in the assigned treatment tank.

At the conclusion of the 90-day exposure period all animals were anesthetized in an overdose of tricaine mesylate (MS-222) (0.4g/L with 1.0g/L bicarbonate buffer) and final length and mass data were collected. Animals were euthanized with the severance of the spine and then placed in cryo-tubes for flash freezing in liquid nitrogen. Those samples were transferred to a -80°C freezer to await further analysis.

Respirometry: Respirometry is a common means to measure the physiological impact of a stressor. We used a closed system respirometer custom-constructed of PVC pipe and a Vernier optical dissolved oxygen probe to measure oxygen consumption as a proxy for metabolic rate. Prior to the start of the experiment, the respirometer was run without a lamprey to determine if background oxygen consumption (either by microbial activity or by diffusion into the chamber itself) could be detected. No background oxygen consumption was detected confirming that any measured change in oxygen within the chamber containing a lamprey was the result of the lamprey's aerobic activity. Each lamprey was evaluated individually, and data were analyzed using 9 to 12 lamprey per treatment (3-4 per replicate). Prior to the start of an experiment, each larva was fasted for > 24h. Each animal was removed from its container, blotted dry with a damp sponge, and its volume determined via displacement of water in a graduated cylinder. The mass of the animal was measured on an analytical balance. These two values, volume and mass, combined with oxygen uptake rate, were used to calculate mass-specific metabolic rate. This methodology was adapted from Sevendsen et al (2016).

Each animal was then placed in the respirometer with flow-through water to acclimate for 45 minutes. At the end of the 45-minute acclimation period, the oxygen

probe was then inserted, thereby sealing the tube. The probe relays temperature and oxygen readings via Bluetooth to a laptop and oxygen consumption was measured for a 10-minute period. At the end of the 10-minute measurement period the probe was removed, allowing fresh water to flow in the tube. The animal was then rested for two minutes before replicating the process for a total of three times per animal at one temperature. At the end of the experiment, each animal was restored to its original container and tank. Note that MS-222 was not used during handling as its use would affect the metabolism of the animal (Oikawa et al 1994).

RNA Sequencing: To complement our physiological growth response data, we quantified changes in the rates of gene expression (differential expression; DE) associated with a change in water temperature using next generation RNA sequencing.

Five lamprey each from the 13° and 25°C treatments, plus four lamprey each from the 15°, 20°, and 22.5°C treatments were sampled for RNA processing. The tissue used was predominantly white muscle from the tail as it was the most abundant tissue available. RNA was extracted using using the Direct-zol RNA Extraction Kit (ZYMO Research, CA). Additionally, this step was used as a quality control to ensure that the RNA integrity number was ≥ 7. Once RNA extraction was complete and QC was performed samples were sent to the OSU Center for Genome Research & Biocomputing (CGRB) for RNA processing.

Using DESeq2 software and a reference genome for Sea lamprey (SIMRbase Genome), we worked to identify specific genes that were differentially expressed in reponse to elevated temperature exposure. Due to the low alignment between our

samples and the Sea lamprey reference genome there is a high likelihood that the data captured in this study only represent a small part of the true DE. However, due to the lack of an annotated Pacific lamprey genome at the time of this study, the surrogate Sea lamprey genome was as close a match as we could find and has been used previously for similar genetic alignment methodologies (Hess et al 2013).

Functional enrichment analysis was performed using gProfiler (version e100_eg47_p14_7733820) with QuickGo (version 2020-07-31) used to create the Gene ontology trees. Enrichment analysis takes the DE and groups them into hierarchical bins based on the known function of said genes. This tool allowed us to determine if the genes being differentially expressed are the result of random chance or were responding to a stimuli, in this case, heat stress.

Statistics: Growth and metabolism data were analyzed by ANOVA followed by Tukey Honestly Significant Difference (HSD) tests. DESEQ2 contrast was used to analyze the DE data with a significance value set at $p<0.1$. Mortality differentials were statistically evaluated using Fisher's exact test (p-value<0.5). Functional enrichment analysis used Bonferroni correlation (p-value<0.05).

RESULTS

Mortality was seen in all treatments and while the trend points to an increase in mortality at higher temperatures there was not a statistically significant difference in mortality among treatments (Fisher's exact test ($p > 0.05$; Table 1).

Table 1: The number and % mortality seen in each temperature treatment over the 90d experiment.

Treatment (°C)	Survived	Died	% Mortality
13	57	4	6.6
15	47	2	4.1
20	44	4	8.3
22.5	31	5	13.9
25	28	5	15.2

Length changed significantly across temperature treatments, with increased loss of length at higher temperatures (Figure 1). Mass changed significantly across temperature treatment, with greater weight loss at higher temperatures (Figure 2).

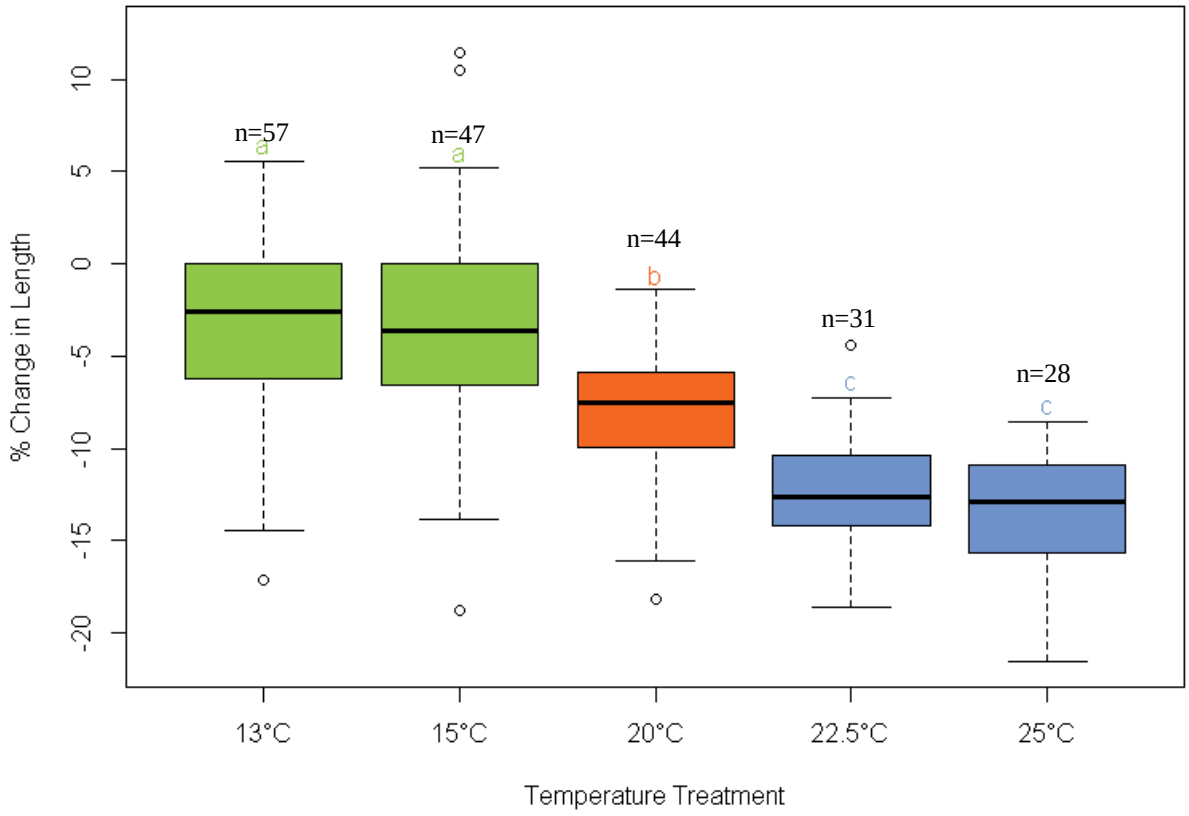

Figure 1: The percent change in length for larval lamprey held at each of five temperature treatments over a period of 90 days. N = 28 to 57 individuals for each temperature treatment. Color and letters indicate statistically distinct groups based on the results of the Tukey HSD test ($p < 0.05$). Each box spans the interquartile range (IQR) and shows the median as a bold line. Whiskers encompass data that are within 1.5-times the IQR of each quartile, beyond which data are shown as circles. We plot the pooled data for individuals because there was no effect of tank (n=3 per treatment) on change in length.

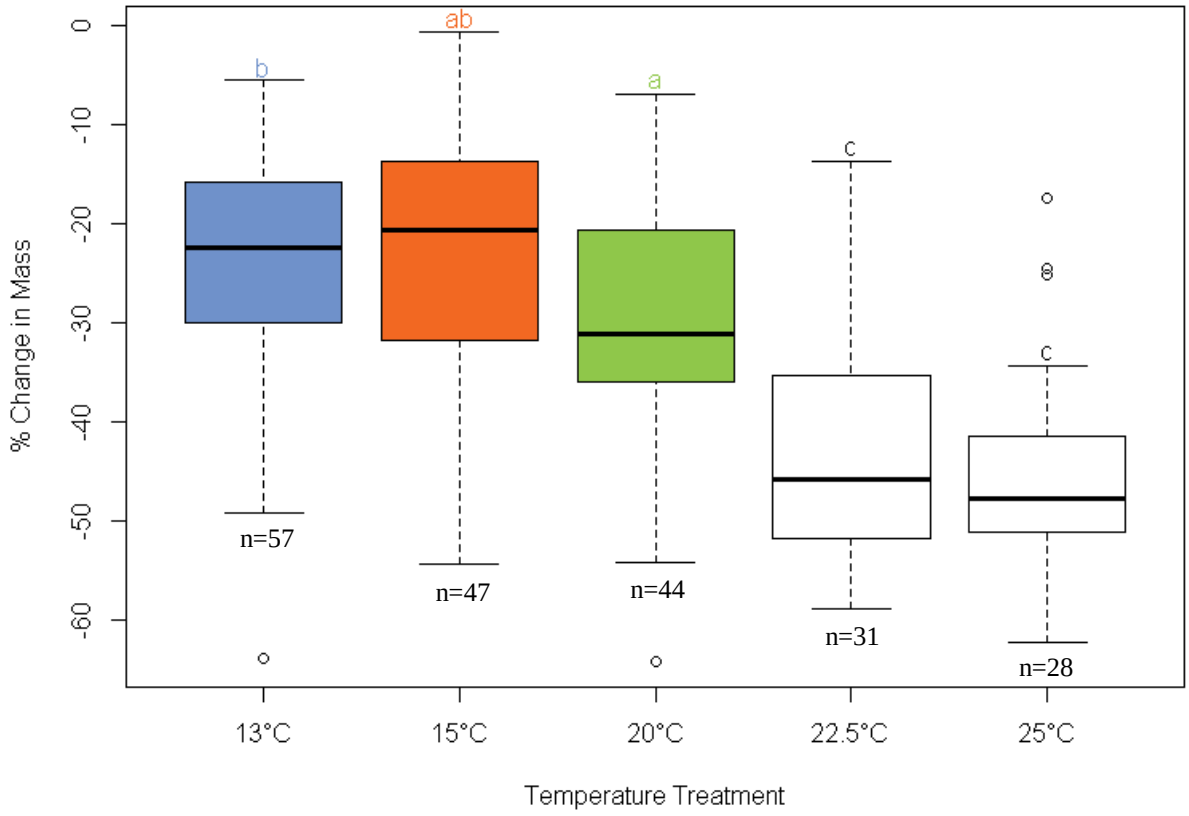

Figure 2: The percent change in mass for larval lamprey held at each of five temperature treatments over a period of 90 days. N = 28 to 57 for each temperature treatment. Color and letters indicate statistically distinct groups based on the results of the Tukey HSD test ($p < 0.05$). Each box spans the interquartile range (IQR) and shows the median as a bold line. Whiskers encompass data that are within 1.5-times the IQR of each quartile, beyond which data are shown as circles. We plot the pooled data for individuals because there was no effect of tank (n=3 per treatment) on change in length.

Standard metabolic rate differed across temperature treatments significantly with standard metabolic rate increasing as temperature increased (Figure 3).

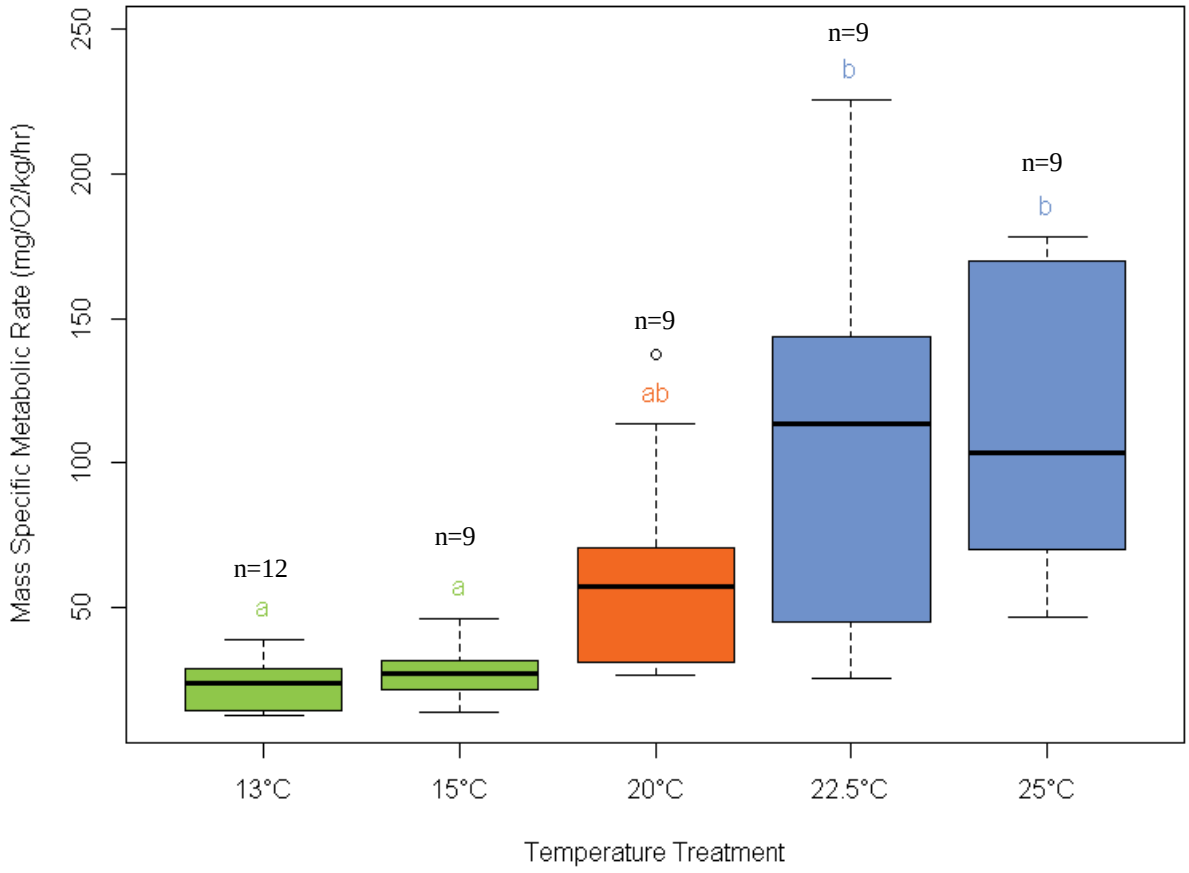

Figure 3: Mass-specific metabolic rate for larval lamprey held at each of five temperature treatments over a period of 90 days. Each treatment had N = 9 except 13°C, which had N = 12. Color and letters indicate statistically distinct groups based on the results of the Tukey HSD test ($p < 0.05$). Each box spans the interquartile range (IQR) and shows the median as a bold line. Whiskers encompass data that are within 1.5-times the IQR of each quartile, beyond which data are shown as circles. We plot the pooled data for individuals because there was no effect of tank (n=3 per treatment) on change in length.

Temperature had a significant effect on DE (Figure 4), with the largest DE seen between 15° and 25°C at a total of 230 differentially expressed genes (Table 2). Results of the functional enrichment analysis indicate no significant activation of the heat shock response and instead, the gene pathways involving growth suppression, protein sorting and protein catabolism were enriched (appendix table 1).

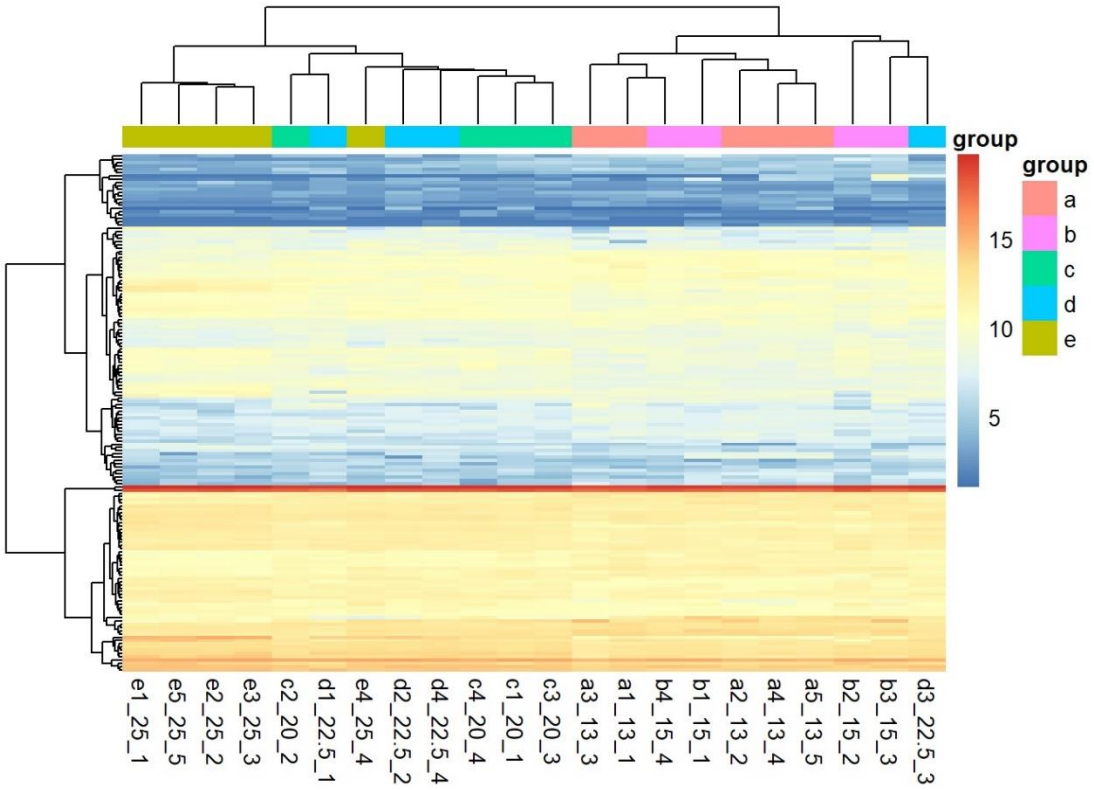

Figure 4: Heat map describing the rate of differential expression of individual genes from each lamprey at each of five temperature treatments over a period of 90 days. The x-axis denotes the individual lamprey with the ID indicating the source treatment. Group a=13°C, b=15°C, c=20°C, d=22.5°C, and e=25°C. The brackets on the y-axis group genes based on similar levels of expression of a specific gene. The brackets on the top group individuals by the overall similarity in their rates of gene expression.

Table 2: The total number of differentially expressed genes across treatments.

TEMP(°C)	13	15	20	22.5	25
13	0	22	59	50	158
15		0	94	61	230
20			0	12	23
22.5				0	12
25					0

DISCUSSION

We hypothesized that growth rate would exhibit a dome-shaped response to warming as temperatures approached and exceeded the physiological optimum (Figure 5). However, we instead observed a solely negative effect of temperature on growth (Figures 1 & 2). Temperature increased basal metabolism (Figure 3), and thus rates of energy expenditure, which is consistent with our current understanding of how poikilothermic organisms respond to temperature. When food rations are high, temperature can increase growth by increasing assimilative capacity. However, if rations are low (and assimilative capacity is not limiting) or feeding stops, then warming increases energetic costs without increasing gains (Brett 1969), thereby reducing growth. Under starvation, growth is always negative and mass loss increases with temperature. Thus, it appears the fish in our study were not feeding at levels high enough to maintain mass. Alternatively, it is possible that fish in our treatments fed, but all of our temperature treatments exceeded the thermal optimum for lamprey. This is unlikely because our coldest treatment, 13°C, was ~10°C lower than the maximum annual temperature experienced by our focal population, yet still generated negative growth. Even coldwater specialists such as bull trout (*Salvelinus confluentus*), exhibit positive growth at 13°C when fed *ad libitum* (Mesa et al 2013, Selong et al 2001).

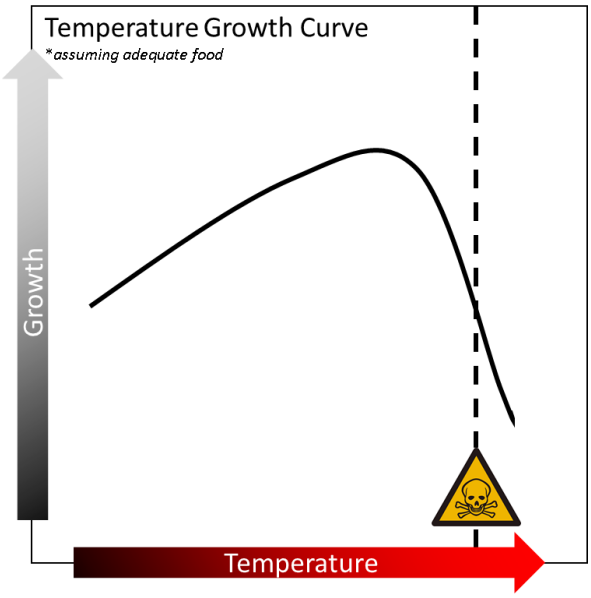

Figure 5: Hypothesized curve of the effect of temperature on the growth of larval lamprey.

While the apparent lack of feeding during the experiment precluded characterization of the scope for growth, it provided an opportunity to test the tolerance and genetic response of lamprey to simultaneous food deprivation and high temperatures. Temperature and starvation should have synergistic negative effects on energy balance that would be challenging for fish to sustain over seasonal time scales. There was some indication that under food limited conditions, mortality may increase with increasing temperature (although this failed to reach statistical significance), but mean survival was always above 85%, revealing a high capacity to withstand energy loss over an extended period of time (90 days). It is possible the high lipid content of lamprey, which are about 3-times more energy dense than salmonids, provides them the caloric capacity to endure starvation (Whyte et al 1993). In the wild, lamprey rely on lipid stores to fuel metamorphosis into parasitic juveniles, which entails a 4-5 month period of starvation (McGee et al 2008). Our results suggest that lamprey can

withstand an extended period of exogenously-driven starvation. This may indicate a level of resistance to prolonged sub-optimal temperature and feed conditions.

Our treatments elicited a differential genetic response that varied with increasing temperature but was more indicative of starvation than heat stress. The differentially expressed genes did correlate with the temperature (Figure 4) with the number of DE genes being higher at higher temperature (Table 2). However, based on the functional enrichment analysis, the pathways identified are not part of the thermal/heat shock response, rather, they suggest the larva were catabolizing proteins for energy (Appendix Table 1). These findings are striking similar to Han et al (2012) in which sturgeon larvae were exposed to both heat and starvation stress. They postulated that the larvae where unable to mount an effective heat shock response because the sturgeon where relying on catabolism (breaking down proteins) for energy.

Interpretation of these results may be confounded by several issues. We did not intentionally depress feeding rates, and thus did not isolate this treatment factor from that of temperature. The other issue was the source of the reference genome. Due to the lack of an annotated Pacific lamprey genome, the Sea lamprey reference genome was used as a surrogate having ~ 30% alignment with the sample in this project. It could have been the case that the findings of the study may have changed if the genes in the remaining ~70% of the genome were identified.

Taken as a whole, our findings suggest that the larval Pacific lamprey in our study were responding to starvation stress, which inhibited their ability to mount a traditional heat shock response. We documented a significant loss in body length and

mass at all temperature treatments, clearly showing that the animals had been fed below a required maintenance ration. The amount of mass lost was directly linked to the temperature treatment to which the larvae were exposed. However, the genetic finding indicate that pathways associated with starvation, not heat shock, made up the bulk of DE genes. And yet, mortality was relatively low and did not differ significantly between temperature treatments (though this could have with a larger sample size). This study shows that larval Pacific lamprey have a capacity to resist metabolic and heat stress simultaneously for prolonged time frames lasting entire seasons if need be.

These findings also suggest new avenues for future research, including how the acute and chronic stress responses differ. While we were able to simulate starvation in a lab, it is unknown if food is a limiting factor in the wild for filter feeders like larval lamprey. Finally, while this study shows the capacity of stress resistance in larval Pacific lamprey, it is unknown whether fish in the wild could sustain this level of energy depletion without incurring subsequent loss of fitness. Poor growth can result in carry-over effects such that performance at a later life stage may ultimately reflect experience in prior seasons and habitats.

CONCLUSION

Pacific lamprey are an important part of the cultural and ecological heritage of the western coast of North America. Yet compared to salmonids, relatively little is known about their physiological response to environmental stress. Our results suggest that these animals are capable of resisting the combined stress of food deprivation and elevated water temperature, showing high survival across a range from 13°-25°C, for 90 days. However, the capability to resist stressors does not necessarily reflect resilience as there may have been lingering negative impacts on the survival and fitness of these animals later in life. Questions that remain, include the long term sub-lethal effects of chronic stress, the interaction between other common environmental stressors, and the possible behavioral responses not available in this study. Overall this work reveals that larval Pacific lamprey may have a robust capacity to ameliorate chronic stressors at the cost of growth.

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APPENDICES

Appendix Table 1: Comprehensive list of ontological terms up/down regulated in treatment comparisons. GO Term can be searched in most online ontology libraries. Gene ontology abbreviations (BP=Biological Process, CC=Cellular Component, MF=Molecular Function).

Treatment	GO Term	Term Name	UP or DOWN Reg.	Gene Ontology
13 v 15	GO:0071985	multivesicular body sorting pathway	UP	BP
	GO:0000814	ESCRT II complex	UP	CC
	GO:0005768	endosome	UP	CC
	GO:0010008	endosome membrane	UP	CC
	GO:0036452	ESCRT complex	UP	CC
	GO:0006914	autophagy	DOWN	BP
	GO:0061919	process utilizing autophagic mechanism	DOWN	BP
13 v 20	GO:0035226	glutamate-cysteine ligase catalytic subunit binding	UP	MF
	GO:0016933	extracellularly glycine-gated ion channel activity	DOWN	MF
	GO:0016597	amino acid binding	DOWN	MF
	GO:0016594	glycine binding	DOWN	MF
13 v 22.5	GO:0008537	proteasome activator complex	UP	CC
	GO:0005488	binding	DOWN	MF
	GO:0051082	unfolded protein binding	DOWN	MF
	GO:0005832	chaperonin-containing T-complex	DOWN	CC
	GO:0101031	chaperone complex	DOWN	CC
13 v 25	GO:0016853	isomerase activity	UP	MF
	GO:0065008	regulation of biological quality	UP	BP
	GO:0005488	binding	DOWN	MF
	GO:0051082	unfolded protein binding	DOWN	MF
	GO:0006457	protein folding	DOWN	BP
15 v 20	GO:0022627	cytosolic small ribosomal subunit	UP	CC
	GO:0004017	adenylate kinase activity	DOWN	MF
	GO:0015030	Cajal body	DOWN	CC
	GO:0016604	nuclear body	DOWN	CC

Table 1 Cont’				
15 v 22.5	GO:0003735	structural constituent of ribosome	UP	MF
	GO:0005198	structural molecule activity	UP	MF
	GO:0006412	translation	UP	BP
	GO:0043043	peptide biosynthetic process	UP	BP
	GO:0043604	amide biosynthetic process	UP	BP
	GO:0006518	peptide metabolic process	UP	BP
	GO:0043603	cellular amide metabolic process	UP	BP
	GO:1901566	organonitrogen compound biosynthetic process	UP	BP
	GO:0044267	cellular protein metabolic process	UP	BP
	GO:0019538	protein metabolic process	UP	BP
	GO:0044271	cellular nitrogen compound biosynthetic process	UP	BP
	GO:0034645	cellular macromolecule biosynthetic process	UP	BP
	GO:0009059	macromolecule biosynthetic process	UP	BP
	GO:1901564	organonitrogen compound metabolic process	UP	BP
	GO:0010467	gene expression	UP	BP
	GO:0008152	metabolic process	UP	BP
	GO:0044260	cellular macromolecule metabolic process	UP	BP
	GO:0044249	cellular biosynthetic process	UP	BP
	GO:1901576	organic substance biosynthetic process	UP	BP
	GO:0009058	biosynthetic process	UP	BP
	GO:0044237	cellular metabolic process	UP	BP
	GO:0034641	cellular nitrogen compound metabolic process	UP	BP
	GO:0005840	ribosome	UP	CC
	GO:0043228	non-membrane-bounded organelle	UP	CC
	GO:0043232	intracellular non-membrane-bounded organelle	UP	CC
	GO:0005622	intracellular	UP	CC
	GO:0043229	intracellular organelle	UP	CC
	GO:0043226	organelle	UP	CC
	GO:0022627	cytosolic small ribosomal subunit	UP	CC
	GO:0008537	proteasome activator complex	UP	CC
	GO:0006606	protein import into nucleus	DOWN	BP

Table 1 Cont’				
	GO:0034504	protein localization to nucleus	DOWN	BP
	GO:0051170	import into nucleus	DOWN	BP
	GO:0017038	protein import	DOWN	BP
	GO:0006913	nucleocytoplasmic transport	DOWN	BP
	GO:0072594	establishment of protein localization to organelle	DOWN	BP
	GO:0051169	nuclear transport	DOWN	BP
	GO:0033365	protein localization to organelle	DOWN	BP
	GO:0031080	nuclear pore outer ring	DOWN	CC
15 v 25	GO:0003735	structural constituent of ribosome	UP	MF
	GO:0005198	structural molecule activity	UP	MF
	GO:0003674	molecular_function	UP	MF
	GO:0016491	oxidoreductase activity	UP	MF
	GO:0003824	catalytic activity	UP	MF
	GO:0016853	isomerase activity	UP	MF
	GO:0055114	oxidation-reduction process	UP	BP
	GO:0043603	cellular amide metabolic process	UP	BP
	GO:0006412	translation	UP	BP
	GO:0043043	peptide biosynthetic process	UP	BP
	GO:0043604	amide biosynthetic process	UP	BP
	GO:0006518	peptide metabolic process	UP	BP
	GO:0008152	metabolic process	UP	BP
	GO:0005840	ribosome	UP	CC
	GO:0043228	non-membrane-bounded organelle	UP	CC
	GO:0043232	intracellular non-membrane-bounded organelle	UP	CC
	GO:0006606	protein import into nucleus	DOWN	BP
	GO:0034504	protein localization to nucleus	DOWN	BP
	GO:0051170	import into nucleus	DOWN	BP
20 v 22.5	GO:0031012	extracellular matrix	DOWN	CC
20 v 25	GO:0008568	microtubule-severing ATPase activity	UP	MF
	GO:0051013	microtubule severing	UP	BP
	GO:0000922	spindle pole	UP	CC

Table 1 Cont’				
	GO:0005819	spindle	UP	CC
22.5 v 25	GO:0008568	microtubule-severing ATPase activity	UP	MF
	GO:0051013	microtubule severing	UP	BP
	GO:0000922	spindle pole	UP	CC
	GO:0005819	spindle	UP	CC
	GO:0006914	autophagy	DOWN	BP
	GO:0061919	process utilizing autophagic mechanism	DOWN	BP

Appendix Table 2: Full list of the lamprey temperatures, ID, tank number, with pre and post length and mass sampling. Portion of table in white indicates first 90d experiment; portion of table in grey indicates second 90d experiment.

Temp (°C)	Fish ID	Tank	Start Length (mm)	Start Mass (mg)	End Length (mm)	End Mass (mg)
13	C3	5	61	446.6	60	276
13	C14	5	65	428.3	63	380.8
13	C5	5	100	1489.8	98	1150.7
13	C9	5	89	1134	89	927.1
13	C7	5	61	496.6	62	366.6
13	C16	5	84	917	79	710.9
13	C4	5	65	461	64	342.2
13	C15	5	67	574.6	65	410.1
13	C8	5	70	588.2	66	370.1
13	C10	5	108	2100.7	114	1984.5
13	C12	5	70	583.3	70	408.6
13	C13	5	74	665.9	68	338.6
13	C11	5	95	1273.4	97	1173.8
13	C1	5	87	1121.5	85	829.6
13	C2	5	98	1448.2	100	1291.9
13	C46	4	49	194.7	45	144.9
13	C45	4	65	460	62	335.1
13	C43	4	70	590.4	58	213.7
13	C47	4	80	764	72	626.9
13	C44	4	70	590.4	72	517.4
13	C42	4	65	503.1	68	385.7
13	C39	4	80	721.3	75	603.3
13	C35	4	58	367.7	54	238
13	C48	4	68	487.3	67	384.7
13	C34	4	62	384.6	58	251.8
13	C36	4	65	485.1	60	327.1
13	C41	4	76	708.2	77	634.2
13	C33	4	76	683.4	75	615.6
13	C40	4	64	358.6	60	246
13	C38	4	70	614.5	71	486.3
13	C20	3	64	443.6	63	363.1
13	C25	3	100	1575	100	1432.2
13	C29	3	68	473.3	65	429.1
13	C22	3	97	1437.8	83	1134
13	C31	3	59	331	59	271.9
13	C30	3	78	723.9	76	634.9
13	C19	3	86	1022.3	87	861.3
13	C26	3	72	585.7	71	484
13	C21	3	65	475.1	65	382.2
13	C17	3	80	822.8	81	669
13	C28	3	97	1306.6	72	535.9
13	C18	3	66	548	69	469.8

13	C28	3	97	1306.6	99	1170.6
13	C24	3	71	622.4	70	456.8
13	C27	3	55	247.2	48	168
13	C32	3	71	530.3	65	381.7
15	MT3	2	76	713.6	71	554
15	MT4	2	74	639.7	73	584.8
15	MT14	2	84	827.3	82	644.5
15	MT12	2	95	1281.5	95	1215.5
15	MT16	2	75	681.1	70	456.5
15	MT11	2	82	872.2	80	691.8
15	MT9	2	67	497.4	68	413.5
15	MT1	2	77	796.4	81	791.8
15	MT10	2	144	2721.3	117	2431.9
15	MT13	2	100	1454.5	96	1097.3
15	MT7	2	85	941.4	80	769.2
15	MT2	2	65	411.2	56	199.4
15	MT15	2	88	952.8	89	781.8
15	MT5	2	78	773.4	73	441.8
15	MT8	2	95	1294.4	97	1164.6
15	MT6	2	61	390.6	55	267.2
15	MT27	14	87	1041.8	86	894.5
15	MT23	14	80	783.3	73	527.7
15	MT22	14	84	895.1	75	538.3
15	MT24	14	70	492.8	64	282
15	MT25	14	85	922.5	80	757.6
15	MT17	14	97	1466	93	997.4
15	MT21	14	80	877.8	79	731.5
15	MT29	14	73	551.6	71	445
15	MT26	14	86	916.5	80	684.8
15	MT19	14	87	978.7	87	848.5
15	MT28	14	55	298.8	53	177.1
15	MT31	14	78	635.8	76	582.1
15	MT20	14	74	604.2	70	467.5
15	MT18	14	68	519.5	69	431.3
15	MT32	14	92	1175.9	91	1072.1
15	MT30	14	90	1092.8	90	938.2
15	MT44	10	63	367.4	62	307.1
15	MT34	10	66	490.3	62	347.1
15	MT36	10	105	1891.8	117	1747.3
15	MT37	10	105	1902	116	1815.4
15	MT45	10	71	618.3	71	429.4
15	MT48	10	69	475	69	448.6
15	MT41	10	68	509.8	63	368.6
15	MT33	10	61	303	57	146.2
15	MT35	10	65	445.7	59	247.4
15	MT46	10	89	1038.2	85	742.9
15	MT40	10	66	423.4	62	272
15	MT42	10	70	524.6	61	239.1
15	MT43	10	60	341.4	60	266.6

15	MT47	10	96	1302.7	91	1079.8
15	MT38	10	89	1155.8	90	1020.5
20	HT12	1	47	191.5	40	93.4
20	HT16	1	81	839.6	74	571.4
20	HT11	1	56	297.8	52	196.3
20	HT7	1	60	376.4	57	296
20	HT15	1	61	366.9	56	237.4
20	HT10	1	88	1020.1	84	886.7
20	HT14	1	58	326.4	54	235.2
20	HT4	1	100	1587.8	93	1246.7
20	HT3	1	74	688.7	73	610.9
20	HT8	1	91	1086.5	88	1010.5
20	HT5	1	69	538.7	67	453.7
20	HT9	1	70	552.4	67	436.6
20	HT13	1	90	1070.8	80	623.1
20	HT2	1	68	528.3	62	364.5
20	HT6	1	72	601.5	65	438.5
20	HT1	1	77	785.7	72	626
20	HT37	12	62	381.2	52	136.6
20	HT38	12	67	450.7	61	371.8
20	HT41	12	81	889.7	75	523.1
20	HT42	12	72	596.9	70	520.7
20	HT33	12	78	654	70	431.3
20	HT43	12	70	508.9	64	343
20	HT48	12	77	728.7	70	485.7
20	HT45	12	58	346.5	54	250.3
20	HT35	12	55	246.3	45	112.9
20	HT47	12	86	990.5	79	705.5
20	HT36	12	81	846	74	573.8
20	HT34	12	79	772	73	563.5
20	HT32	13	94	1301.9	90	1073.8
20	HT24	13	79	742.3	69	464.6
20	HT18	13	68	456.4	60	295.6
20	HT31	13	75	603	68	373.3
20	HT28	13	97	1421.9	92	1248.9
20	HT23	13	103	1609.9	96	1112.8
20	HT26	13	78	757.3	73	520.7
20	HT17	13	95	1207.7	89	1053.1
20	HT21	13	80	924.6	78	738.8
20	HT22	13	84	885.2	74	562.2
20	HT30	13	71	568.3	66	369.4
20	HT19	13	63	413.4	56	245.6
20	HT29	13	80	786.4	74	511.5
20	HT20	13	71	610.4	63	375.5
20	HT25	13	56	274.3	53	206.7
20	HT27	13	55	252.3	47	117.8
13	C52	16	97	988.5	87	770.3
13	C53	16	75	590.5	73	442.9
13	C60	16	63	347.4	59	230.4

13	C56	16	64	357.8	60	243.8
13	C54	16	66	423.5	64	324.2
13	C51	16	81	672.4	74	426.3
13	C55	16	76	660.2	73	439
13	C59	16	93	1069.1	90	816.8
13	C77	16	104	1307.9	102	1198.8
13	C49	16	58	293.9	55	197.8
13	C57	16	79	672.9	75	545.8
13	C64	16	122	2542.7	120	2291.4
22.5	MT73	A2	86	777.1	71	518.3
22.5	MT70	A2	61	373.3	53	209.9
22.5	MT78	A2	95	1228.1	83	800
22.5	MT69	A2	81	758.8	70	311.9
22.5	MT63	A2	64	327	56	185
22.5	MT72	A2	69	414.4	64	212.6
22.5	MT65	A2	68	352	58	183.9
22.5	MT77	A2	96	1077	84	786.6
22.5	MT61	A2	59	300.6	50	138.6
22.5	MTc69	A10	70	529.3	64	332.5
22.5	MTc79	A10	99	1073.5	85	691.1
22.5	MT71	A10	67	432.9	60	291.1
22.5	MTc78	A10	75	652.4	66	347.9
22.5	MTc66	A10	59	256.9	51	122.1
22.5	MTc75	A10	79	745.2	68	434.2
22.5	MTc73	A10	78	697.3	70	322.4
22.5	MT50	A10	102	1331.7	87	789.6
22.5	MT55	A10	70	456.3	60	192.9
22.5	MTc62	A10	81	783.6	68	422.1
22.5	MTc70	A10	120	2180.2	107	1476.6
22.5	MT52	A14	101	1277.6	88	994.1
22.5	MT60	A14	59	277.4	48	142.3
22.5	MT62	A14	79	743.4	73	603.2
22.5	MT76	A14	68	424.8	65	217.6
22.5	MT59	A14	78	659.3	70	380.3
22.5	MT80	A14	66	427.7	58	186.4
22.5	MT58	A14	95	1265.5	85	618.1
22.5	MT53	A14	60	295.9	54	160.3
22.5	MT57	A14	61	354	56	151.9
22.5	MT68	A14	115	1719.5	99	1483.4
22.5	MT49	A14	67	401.5	55	175.6
25	HT64	A1	82	680.8	74	513.8
25	HT67	A1	84	782.6	72	403.4
25	HT68	A1	65	388.3	51	158.9
25	HT80	A1	115	2225.7	101.2	1837.5
25	HT65	A1	78	644.2	66	342.2
25	HT62	A1	75	510.5	67	261.5
25	HT63	A1	117	2126.9	105	1593.1
25	HT70	A1	98	1185.3	81	656.8
25	HTc63	A12	67	428.3	55	255.8

25	HT75	A12	76	603.7	69	308.1
25	HT76	A12	73	492.6	64	253
25	HTc65	A12	80	669.1	70	371.8
25	HT73..	A12	87	843.2	75	454.1
25	HT74	A12	70	500	64	206.9
25	HTc61	A12	90	972.6	78	632.5
25	HT61	A12	90	1030	79	516
25	HTc74	A12	75	575.3	63	316.6
25	HT71	A12	57	285.2	50	122.3
25	HT72	A12	93	1226.8	83	804.9
25	HT54	A13	69	430	59	198.9
25	HT57	A13	68	479.7	60	309
25	HT56	A13	69	445.8	58	243.2
25	HT60	A13	99	935.2	79	444.3
25	HT50	A13	82	691.6	70	347.9
25	HT52	A13	85	779.5	73	400.6
25	HT53	A13	60	436.7	54	165.1
25	HT55	A13	72	521.6	64	299.1
25	HTc80	A13	90	919.6	75	413.8