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Predator life history and prey ontogeny limit natural selection on the major armour gene, *Eda*, in threespine stickleback

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Abstract

Natural selection shapes the evolution of antipredator traits in prey. However, selection in the wild depends on ecological context, including features of predator and prey populations, making field studies of selection critical to understanding how predators shape selection on prey defences. Threespine stickleback (*Gasterosteus aculeatus*) is a classic system to study the effects of predators on the natural selection of prey. In lakes and rivers, fish predators have been shown to impose selection against low plated adult stickleback phenotypes and genotypes. We directly measured selection by predatory salmonids on the *Ectodysplasin-A* (*Eda*) gene in estuary stickleback from California. Despite previous studies showing a positive correlation between predator presence and frequency of the *Eda* “complete” allele in estuary populations, we found that *Eda* “low” genotypes were not significantly more frequent in salmonid predator diets. Further, we found no evidence of changes in *Eda* genotype frequencies across generations that would suggest directional selection driven by predators. Prior selection studies have examined the effects of large resident trout on adult stickleback. In contrast, predators in this study were juvenile anadromous salmonids, which only ate juvenile stickleback whose plate phenotypes had not fully developed. Thus, in this case, predator life history and stickleback ontogeny may preclude strong selection on stickleback armour. Our results underscore the importance of selection studies in the wild for understanding the context-dependent nature of selection in natural populations.

KEYWORDS

anadromy, antipredator adaptation, *Ectodysplasin-A* gene, gape limitation, intraspecific variation

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1 | INTRODUCTION

Antipredator traits include some of the most impressive examples of evolution by natural selection. Weapons, armour, toxins, crypsis, and a wide variety of behavioural tactics all evolved to help organisms evade their predators (Vermeij, 1982). Yet natural selection is often context-dependent (Wade & Kalisz, 1990). Differences in the environment or differences between populations of the interacting species may alter the ecology of species interactions and thus the potential for evolutionary responses (Thompson, 1997, 2005). Here we test whether predation of threespine stickleback (*Gasterosteus aculeatus*, Linnaeus, 1758) by predatory fishes is selective in an intermittently open estuary environment, as it has been shown to be in lake and riverine habitats (Bañbura et al., 1989; Hagen & Gilbertson, 1973; Reimchen, 1994).

Inefficient predators (i.e. those that do not capture each prey pursued) can cause natural selection of defence traits in prey (Reimchen, 1994; Vermeij, 1982). Several lines of evidence suggest that armour, including bony lateral plates and spines in stickleback, is selected for by predators, including fish (reviewed in Barrett, 2010; Bell, 2001; Miller et al., 2017). Large differences in plate numbers are found repeatedly between marine and freshwater stickleback populations (Bell & Foster, 1994; Colosimo et al., 2005). Marine populations have a full complement of lateral plates along their entire body and large spines, whereas freshwater populations are variable, but most show reduction in spines, plates, or both (Bell & Foster, 1994). Approximately 80% of variation in lateral plate expression is explained by variation at the *Ectodysplasin-A* (*Eda*) locus, with homozygous “complete” genotypes (CC) having a full complement of plates along their entire body, homozygous “low” (LL) genotypes having just a few plates at the anterior end of the body, and heterozygotes (CL) being either partially or completely plated (Colosimo et al., 2004). Stickleback do not achieve their full complement of bony lateral plates until they reach a standard length of about 30 mm (Bell, 1981). Variation in plate phenotypes and *Eda* genotypes has been found to correspond closely with the suite of coexisting predators (Gross, 1978; Hagen & Gilbertson, 1972; Paccard et al., 2018; Reimchen et al., 2013). Analyses of the diets of predatory fishes including rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) in lakes and northern pike (*Esox lucius*, Linnaeus, 1758) in streams show that less armoured stickleback are more readily eaten by predators (Bañbura et al., 1989; Hagen & Gilbertson, 1973).

However, plate phenotypes and *Eda* genotypes are associated with a variety of other morphological and behavioural traits, complicating efforts to pinpoint the target of selection (Rennison et al., 2015). For example, increasing numbers of plates are associated with decreased burst swimming speed in adult fish (Bergstrom, 2002; Taylor & McPhail, 1986). *Eda* “complete” genotypes are more adept at schooling (Greenwood et al., 2016) and have a different number and arrangement of neuromasts in their lateral line, which is a key sensory organ (Mills et al., 2014). These traits may also contribute to success or failure in surviving encounters with predators and therefore modify selection on *Eda*.

Experimental studies of cutthroat trout (*Oncorhynchus clarkii*, Richardson, 1836) predation on stickleback in lakes have shown the functional role of armour to predator defence (Reimchen, 1983, 1991, 1992, 2000). The anterior plates serve as a buttress between the dorsal and pelvic spines during predator manipulation (Reimchen, 1983, 1992). Posterior plates interfere with swallowing and lead to longer handling time, which means more opportunity for the stickleback to escape (Reimchen, 2000). All plates protect the soft tissue from injuries sustained during failed predation attempts (Reimchen, 1992, 2000). Predation success depends on relative body size of predator and prey (Reimchen, 1991). Stickleback whose effective diameter (including erected spines) was close to or exceeded the gape width of predators were extremely likely to escape and were slightly less likely to even be pursued by the predator (Reimchen, 1992). Posterior plates were especially useful in increasing opportunities for escape when the prey were large relative to predator gape (Reimchen, 2000).

The aforementioned studies of stickleback in lakes and streams support the hypothesis that fish predators can act as a strong agent of directional selection on stickleback plates and the underlying *Eda* gene. However, the nature of that selection may depend on the ecological context, such as the availability of refuge habitat (Leinonen et al., 2011), the types of predators (Reimchen, 1997) or the body size of either predator or prey (Reimchen, 1991, 2000). Stickleback in bar-built estuaries in central California face their predators, including several species of salmonids and sculpins in a different ecological context; yet most work on California stickleback has left out predators and instead focussed on associations between plate morph, *Eda* genotype frequencies, climate and habitat (Baumgartner & Bell, 1984; Des Roches et al., 2020). Bar-built estuaries, or lagoons, are intermittently connected to the ocean due to seasonal rainfall and vary from a flowing, river-like state in winter, to a pond-like state during the summer dry period (Behrens et al., 2013; Rich & Keller, 2013; Williams & Stacey, 2016). Unlike the predominantly monomorphic populations that occur in the Pacific Northwest, stickleback in California estuaries are polymorphic for the *Eda* alleles leading to variation in plate morph (Baumgartner & Bell, 1984; Des Roches et al., 2020; Hagen & Gilbertson, 1973; Paccard et al., 2018). Increased plate numbers and *Eda* “complete” allele frequencies are associated with the presence of fish predators in bar-built estuaries (Paccard et al., 2018; Wasserman et al., 2020). However, those studies did not directly observe fish consuming stickleback, and we therefore do not know how common fish predation is or how fish predation at different life stages acts on natural selection on stickleback in California estuaries. Our focal estuary is south of the range of anadromous stickleback and thus contains a resident population with an *Eda* polymorphism, not a mixture of resident and anadromous fish (Howe, 1973; Paccard et al., 2018).

The salmonid predators in bar-built estuaries include coho salmon (*Oncorhynchus kisutch*, Walbaum, 1792) and steelhead trout (*Oncorhynchus mykiss*). The common name steelhead typically refers to the anadromous form of *O. mykiss*, but in central California, life history is variable. While most individuals are anadromous, some

complete their life cycle entirely in the stream and/or estuary without ever entering the ocean (Bond et al., 2008; Hayes et al., 2008; Shapalov & Taft, 1954). Importantly, unlike the cutthroat trout and rainbow trout in other studies of stickleback predation, the salmonid predators in these estuaries are mostly anadromous juveniles, which emigrate from the estuary well before reaching their adult size (Hagen & Gilbertson, 1973; Hayes et al., 2008; Moodie, 1972; Shapalov & Taft, 1954).

We investigated the effect of predator-induced selection in this novel ecological context by comparing the *Eda* genotype frequencies of live-caught stickleback to stickleback recovered from the diets of predatory salmonids. After determining the selectivity of predators, we ask whether there were any changes in *Eda* genotype frequency observed in the population across generations. Finally, we assess the effects of predator and prey body size on prey consumption.

2 | MATERIAL AND METHODS

We conducted this study in Scott Creek estuary, the terminus of a short, coastal river system on the central California coast, USA (37.0404N, 122.2297W). In May 2015, we captured 121 adult stickleback from the parent generation using a combination of minnow traps (3.0 mm mesh) and small beach seines (3.0 m long × 1.2 m deep, 9.5 mm mesh). Forty-nine predated juvenile stickleback were identified from the stomachs of 344 juvenile steelhead and 147 coho salmon captured during monthly surveys of the lower Scott Creek estuary from June to December 2015. Salmonids were collected using a large beach seine (35 m long × 2.0 m deep [8.0 mm mesh], with a 2.0 m² bag [4.0 mm mesh]), identified to species, measured for fork length (mm) and given a passive integrated transponder (PIT) tag as described by Osterback et al. (2018). We performed gastric lavage on a subset of individuals to remove stomach contents. Gastric lavage is a nonlethal technique to collect stomach contents from live fish, whereby a weak stream of water is introduced to the stomach via a tube to irrigate the stomach cavity and dislodge consumed material out through the mouth. Salmonid fish were anaesthetised using MS-222 prior to gastric lavage and allowed to fully recover before being released back into the wild. Stomach contents were transferred to polyethylene bags, preserved using 95% ethanol, and stored at -13°C until analysis. Lastly, 113 recruited juvenile stickleback were collected on November 10, 2015, during the salmonid survey. Live-caught stickleback (spring parents and fall recruited juveniles) were euthanised with an overdose of MS-222 and frozen until they could be processed. Animal handling protocols were approved by the University of California, Santa Cruz IACUC under protocols Palke-1306, Palke-1310, and Kierj1604A1. Capture of ESA-listed salmonids was authorised by National Marine Fisheries Service under Section 10(a)(1)(A) permit No. 17292-2A. All other animal collections were made pursuant to California Scientific Collector's Permit SC-12752.

In the laboratory, we measured the standard length of live-caught stickleback and preserved a small piece of fin tissue in 95%

ethanol. We counted the left lateral plates of each live-caught stickleback under a dissecting microscope. The stomach contents of each salmonid predator were likewise examined under magnification, and all diet items were sorted, identified to the lowest possible taxonomic unit and enumerated. We collected tissue samples from the subset of consumed stickleback for which there was sufficient tissue (46 out of 49 stickleback) to preserve in 95% ethanol that included both bone and soft tissue when possible. We were unable to identify and count lateral plates on consumed stickleback due to their state of digestion.

The presence of stickleback parts that could not be reliably associated with an individual (e.g. loose spines) was not included in our estimate of the number of fish consumed, given unique specimen IDs, or genotyped. We measured or estimated the standard length of predated juvenile stickleback, except for 3 individuals which were too incomplete to do so.

To extract DNA from unique specimens (both adult and juvenile stickleback), we placed the tissue sample from each specimen into a microcentrifuge tube using forceps sterilised with 50% ethanol. Chelex[®] 100 Resin (Bio-Rad, USA), which binds to Mg²⁺ for DNases, was used to extract genomic DNA from the samples. To prepare the Chelex slurry, 5 g of Chelex Resin and 50 ml of water were added into a conical centrifuge tube, of this, 400 µl of the Chelex slurry was then pipetted into each tube. Each tube was then vortexed (Fisher Vortex Genie 2[™]; Scientific Industries, USA) for approximately 10 s and spun for 5–10 s at high speed in a centrifuge (Eppendorf[®] centrifuge 5415 D, #037001672; MilliporeSigma, USA) until the centrifuge reached 5917.35 rcf. The samples were incubated for 20 min at 95°C in a water bath (Isotemp 205; Fisher Scientific[™], USA). The vortex and centrifuge steps were repeated afterwards to separate the Chelex Resin and extracted DNA.

The marker *Stn382* was used to identify the *Eda* genotype and amplified using polymerase chain reaction (PCR) (Colosimo et al., 2005). The PCR solution was composed of 1 µl of the supernatant from each sample extraction pipetted into PCR tubes with 5 µl of GoTaq[®] Green Master Mix, 2× (Promega, USA, #M7122), 1 µl of 5 µM forward primer (*Stn382f*), and 1 µl of 5 µM reverse primer (*Stn382r*), 1 µl of distilled water, and 1 µl of Bovine Serum Albumin (20 mg/ml, Thermo Scientific[™], #B14). We amplified each sample using a PCR machine (GeneAmp[®] PCR System 9700, #017001106, PE Applied Biosystems, USA) with the cycling conditions starting at 95°C for 4 min, immediately followed by 35 cycles of 30 s at 95°C, 45 s at 55°C and 45 s at 72°C, afterwards they were held at 72°C for 10 min until finally kept cool at 4°C. The PCR products were visualised on 2% agarose gels with GelRed[®] (Biotium, USA) and a 100 bp size standard (Thermo Fisher Scientific, USA) by electrophoresis for 60 min. Homozygous “complete” and homozygous “low” genotypes are represented by a single 218 bp allele or 158 bp allele respectively (Zeller et al., 2012). Individuals were scored as heterozygous when both the “low” and “complete” bands were present. We repeated the extraction and PCR process for fin clips from 5 steelhead from this population as a negative control. We were only able to amplify DNA from 76 spring adults, 39

predated juveniles and 45 fall recruited juveniles. Since our live-caught samples suffered from poor DNA preservation, we conducted a Kolmogorov–Smirnov test to compare the distributions of lateral plate count (the primary phenotypic effect of *Eda* genotype) distributions of fish whose DNA amplified versus those who whose DNA did not amplify in those samples. However, within both the spring adults and fall recruited juvenile samples, lateral plate count distributions of fish whose DNA amplified versus those who whose DNA did not amplify were similar (Kolmogorov–Smirnov tests; Spring: $D = 0.10$, $p = 0.92$, Fall: $D = 0.24$, $p = 0.09$), and we therefore consider the genotype distributions to be a representative sample.

To determine if salmonids preyed selectively on stickleback, we used a chi-squared test of independence to compare genotype frequencies between spring parents and predated juveniles. If salmonids were selectively preying on stickleback, then we would expect the spring parents to differ from the predated juveniles, because the starting population of juveniles that were born are expected to have the same distribution as their parents (Barrett et al., 2008). We recovered fewer predated individuals than we had expected based on previous studies of salmonid predation on stickleback (e.g. Hagen & Gilbertson, 1973; Reimchen, 1990), so we conducted a power analysis to determine if we had sufficient sample sizes to detect differences in genotype frequencies between groups. Given our sample size of 115 genotyped individuals, 2 degrees of freedom, and significance threshold $\alpha = 0.05$, the comparison of spring parents to predated juveniles had a 99.9% chance of detecting a large effect (Cohen's $w = 0.5$), an 82.9% chance of detecting a medium effect ($w = 0.3$) and a 14.6% chance of detecting a small effect ($w = 0.1$) (Cohen, 1988).

To determine whether genotype frequencies changed between generations, we used a chi-squared test of independence to compare genotype frequencies between spring parents and fall recruited juveniles. If genotype frequencies changed between spring parents and fall recruited juveniles, then that could represent a response to selection on *Eda* from all sources, not just salmonid predators. Given our sample size of 121 genotyped individuals, 2 *df*, and significance threshold $\alpha = 0.05$, the comparison of spring parents to fall recruited juveniles had a 99.9% chance of detecting a large effect ($w = 0.5$), an 84.9% chance of detecting a medium effect ($w = 0.3$) and a 15.2% chance of detecting a small effect ($w = 0.1$) (Cohen, 1988).

Since salmonid predation of stickleback has previously been shown to be gape-limited (Reimchen, 1990, 1991), we conducted two tests for each predator species to understand the effect of predator and prey body size on prey consumption. If an individual predator's diet was sampled on more than one occasion and it never ate stickleback, we used the mean of that individual predator's length measured at each capture. No individual predators were found to consume stickleback on more than one occasion. For individual predators that consumed stickleback on one occasion and were also encountered not consuming stickleback on other occasions, we only used the length measured when that individual was encountered

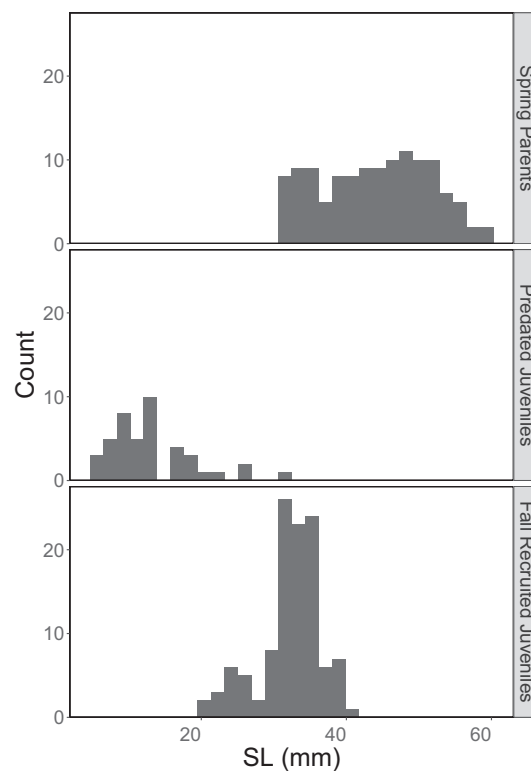


FIGURE 1 Histograms of standard length (mm) of stickleback from the three collections: spring parents, predated juveniles and fall recruited juveniles

consuming stickleback. First, we used Wilcoxon rank sum (WRS) test to compare the lengths of individual predators that consumed stickleback to those that did not because the length of predators was not normally distributed. Second, we regressed prey length against predator length. Our analyses were conducted in R version 4.0.0 and utilised the *pwr* Package version 1.3-0 and the *XNomial* Package version 1.0.4 (Champely, 2020; Engels, 2015; R Core Team, 2020).

3 | RESULTS

Based on length (SL) distributions of collected individuals, the spring stickleback were largest (mean = 44.0 mm, standard deviation [SD] = 7.4 mm), followed by the fall stickleback (mean = 32.3 mm, SD = 4.3 mm), and lastly by the predated stickleback (mean = 13.2 mm, SD = 5.8 mm) (Figure 1). Size distribution differences among groups conform to the notion that the spring sample represented breeding adults, the predated fish were primarily juveniles, and that the fall sample represented recruited juveniles born during spring and summer of 2015 (Raeymaekers et al., 2014; Snyder & Dingle, 1989).

In the spring parents sample, heterozygote was the most common genotype and homozygous complete was the least common genotype (Figure 2). In the predated juveniles, homozygous low was the most common genotype, followed by heterozygote, and then by homozygous complete (Figure 2). *Eda* genotype frequencies of the

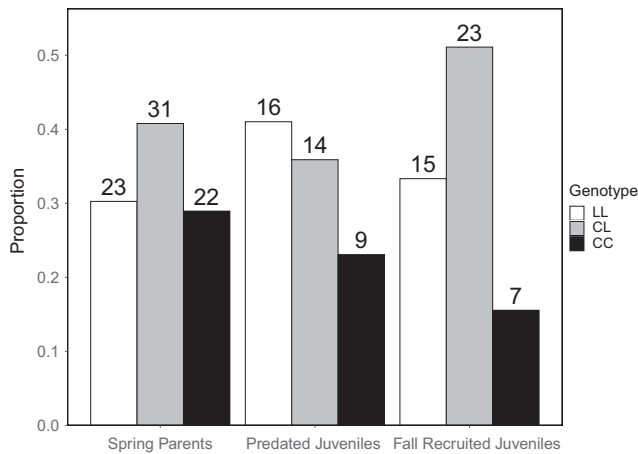


FIGURE 2 Bar charts of *Eda* genotype frequencies of stickleback in the three collections: spring parents, predated juveniles and fall recruited juveniles. Sample sizes are listed above each bar. Genotypes: LL-homozygous low, CL-heterozygous, CC-homozygous complete

predated juveniles were not significantly different from spring parents ($\chi^2 = 1.37$, $df = 2$, $p = 0.51$), indicating no selection by salmonids.

In the fall recruited juvenile sample, heterozygote was the most common genotype and homozygous complete was the least common genotype (Figure 2). *Eda* genotype frequencies of the fall recruited juveniles were not significantly different from spring parents ($\chi^2 = 2.87$, $df = 2$, $p = 0.24$), indicating no evolution from one generation to the next.

We recovered 27 stickleback from the stomachs of juvenile coho salmon and 22 stickleback from the stomachs of juvenile steelhead. While all of the 147 coho salmon stomach samples examined contained discernible prey items (i.e. were nonempty), only 11 (7.5%) contained stickleback. Juvenile coho salmon that consumed stickleback contained between one and 15 individuals (mean = 2.5 stickleback, $SD = 4.2$). Stickleback were found to be a prey item of coho salmon during all months from July to November. By contrast, only 10 of 344 (2.9%) juvenile steelhead stomachs examined contained stickleback. Steelhead that had eaten stickleback consumed between one and six individuals (mean = 2.2 stickleback, $SD = 1.5$). Steelhead with stickleback in their stomachs were encountered during all months between July and December, except September. Only two predated juveniles were recovered in December. While these two fish are from after the date of our fall recruited juvenile sampling, we retained them since they were still juveniles at the time they were eaten.

Consistent with the idea of gape limitation, predator size predicted stickleback consumption. Coho salmon that ate stickleback were larger than those that did not eat stickleback (WRS test, $W = 235$, $p = 0.002$); the median difference between coho samples that ate stickleback and those that did not was 19 mm (95% confidence interval: 7–81 mm), (Figure 3a). Steelhead that ate stickleback were also larger than those that did not eat stickleback (WRS test, $W = 629$, $p = 0.004$); the median difference between steelhead samples that ate stickleback and those that did not was 47 mm (95%

confidence interval: 17–84 mm), (Figure 3b). Predator body size was significantly related to prey body size for coho salmon (Linear Regression, $F_{1,22} = 13.04$, $p = 0.002$, $R^2 = 0.37$), but not for steelhead (Linear Regression, $F_{1,17} = 0.0175$, $p = 0.896$, $R^2 = 0.001$), (Figure 3c).

4 | DISCUSSION

We asked whether salmonids in a bar-built estuary in California selectively preyed on stickleback based on their genotype at the major armour gene *Eda*. Genotype frequencies of predated juveniles were not significantly different from the genotype frequencies in the parental generation, suggesting that salmonids were not selective with respect to *Eda*. The predators (juvenile anadromous salmonids) were smaller than those in previous studies of salmonid predation on stickleback (Moodie, 1972; Reimchen, 1990, 1994). Probably due to gape limitation, the stickleback consumed were relatively small juveniles. Thus, the migratory life history of the salmonid predators (juveniles rearing in the estuary before migrating to sea) constrained them to consuming juvenile stickleback, which did not yet have fully developed plate phenotypes. Consistent with the lack of predator selectivity, we found that the *Eda* genotypes of juvenile stickleback recruiting into the population in the fall did not significantly differ from their parents caught in the spring. These results suggest that salmonids in this environment were not a major selective driver for stickleback plates during the year of our study and that no evolutionary change occurred at the *Eda* locus between stickleback generations.

Without enough developmental time to fully form their plates, the *Eda* “complete” allele appears unable to aid in predator defence. While stickleback in this population grow larger than 60 mm SL (Paccard et al., 2018), the predated stickleback were all juveniles or subadults ≤ 32 mm SL. The majority of predated juveniles were below 14 mm SL, the size at which complete morphs begin to show more plates than low morphs (Bell, 1981). Even if salmonids selected on plate number in these juveniles, that would not translate directly into selection on *Eda* genotype because individuals with similar plate numbers would not reliably have the same genotype.

Juvenile stickleback were most likely to be predated upon, probably because the salmonids in the estuary are mostly smaller than those in previous studies (e.g. Moodie, 1972; Reimchen, 1990). Cutthroat trout that predated on stickleback in Drizzle Lake (British Columbia, Canada) ranged from 120–410 mm standard length (Reimchen, 1990, 1994) and those that predated on stickleback in Mayer lake ranged from approximately 170–400 mm standard length (Moodie, 1972). The vast majority of salmonids that consumed stickleback in previous studies were greater than 200 mm standard length (Moodie, 1972; Reimchen, 1990). In contrast, all of the coho salmon and 86% of the steelhead in our study were less than 200 mm fork length (Figure 3). Most salmonids in our study were smaller than the resident trout of previous studies because they are juveniles of anadromous (coho salmon) or partially anadromous (steelhead) populations, most of which will leave the

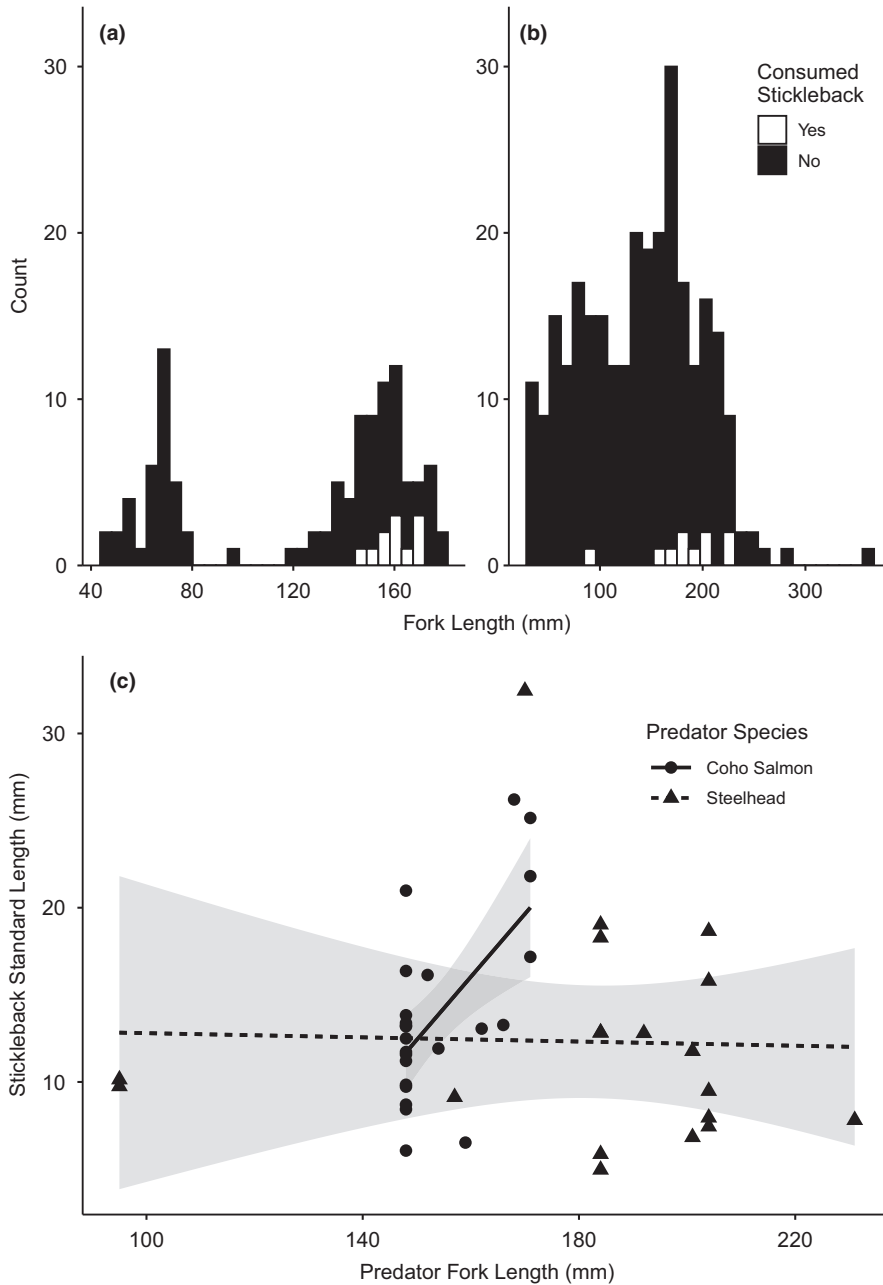


FIGURE 3 Histogram of the size of individual predator fork length for coho salmon (a) and steelhead (b). The relationship between predator fork length and standard length of stickleback consumed. Regression line plus 95% confidence intervals in grey (c)

estuary before reaching their adult size and do not feed on reentry to spawn (Hayes et al., 2008, 2011). Thus, stickleback in Scott Creek rarely face salmonid predators with large enough gape sizes to take adult stickleback, as in the British Columbia lakes (Moodie, 1972; Reimchen, 1990, 1994). Salmonids function as gape-limited predators of stickleback and their ability to consume fish increases with body size (Keeley & Grant, 2001; Moodie, 1972; Reimchen, 1991). In previous studies, there was a significant effect of salmonid predator size on the size of stickleback consumed (Moodie, 1972; Reimchen, 1990). We found a significant effect of predator size on the size of prey for coho salmon but not for steelhead (Figure 3c). The predators available in our study site appeared to straddle the ontogenetic transition to piscivory (Keeley & Grant, 2001). Individuals of both species that ate stickleback were significantly larger than those that did not (Figure 3). Each salmonid that consumed a stickleback (with

the exception of one 95 mm FL steelhead) was in the upper half of the size distribution for its respective species.

Thus, in this study salmonid predators are unlikely to be able to eat stickleback that are larger than juveniles, which may limit the effectiveness of lateral plates as a defence against salmonids in California estuaries. This, in turn, may preclude strong selection by salmonid predators on *Eda*, since the major phenotypic effect of *Eda*, lateral plates, are not fully manifested in juvenile stickleback (Bell, 1981; Reimchen, 2000). By the time stickleback in our focal population develop their adult plate phenotypes (>30 mm SL), they will have escaped salmonid predation into a size refuge. Indeed, a comprehensive study of sources of stickleback mortality in Drizzle Lake, British Columbia (Reimchen, 1990, 1994) found that larger resident cutthroat trout (range 120–410 mm standard length) were the major predator of stickleback, and approximately 80% of trout

had consumed stickleback, whereas juvenile coho salmon (size range 40–130 mm standard length) were an insignificant predator of stickleback, with less than 1% of coho salmon juveniles having consumed stickleback. However, there could also be other differences between the coho salmon and steelhead in Scott Creek and salmonid predators in previous studies that account for their lack of selectivity, especially if those differences affect foraging efficiency during search, pursuit, and manipulation (Reimchen, 1994; Reimchen et al., 2013).

The *Eda* genotypes of predated juvenile stickleback trended in the direction we predicted, with overrepresentation of homozygous “low” genotypes as compared to the parental generation, though the trend was not significant (Figure 2). Our study measured a single prey population over one year. It is possible that these predators are selective and that our sample sizes were insufficient to detect a significant, but small effect. While selection on lateral plates seems unlikely due to stickleback ontogeny, *Eda* does also impact other traits. Individuals with *Eda* “complete” genotypes are more adept at schooling (Greenwood et al., 2016) and have a different number and arrangement of neuromasts in their lateral line (Mills et al., 2014). These traits may also contribute to success or failure in surviving encounters with predators.

We found no significant difference in *Eda* genotype frequencies between spring parents and fall recruited juveniles (Figure 2) and therefore no evidence of net directional selection on *Eda* in this population during this study. Even if there was a small selective effect of salmonid predators, it could be offset by other selective forces or differences in reproductive success. Previous studies of the first year of life of polymorphic stickleback populations have shown temporal changes in the selection on *Eda* and plates (Barrett et al., 2008; Rennison et al., 2015), and our study effectively averages over those different time periods. Stickleback were a minor prey item in the diets of our salmonid predators (frequency of occurrence = 2.9% to 7.5%). This low frequency of occurrence stands in contrast to other studies where stickleback were reported in 89% of nonempty cutthroat trout stomach samples (Reimchen, 1990). Likewise, Hagen and Gilbertson (1973) reported stickleback as a prey item in 10% of rainbow trout (the same species as steelhead in this study) stomachs during spring and up to 80% during the winter. It is therefore unlikely that selection by salmonid predators directly explains the difference in *Eda* genotypes and plate phenotypes between estuary populations in this region (Paccard et al., 2018; Wasserman et al., 2020). However, sites with salmonids differ from those without salmonids in other ways, including the presence of predatory sculpin (*Cottus* spp., *Leptocottus armatus*). A hypothesis remaining to be tested is whether sculpin, rather than salmonids, are capable of driving selection on stickleback armour in these estuaries.

Ecological context is important for understanding if and how species interactions will lead to selection and evolution (Hatfield & Schluter, 1999; Thompson, 2005). Patterns of geographical variation in antipredator defences in stickleback are often associated with predator presence and assumed to indicate adaptation to divergent predator regimes (Hagen & Gilbertson, 1972; Reimchen et al., 2013). Here we tested directly whether steelhead and coho salmon

selectively preyed upon stickleback based on their *Eda* genotype. Results indicate that juvenile anadromous salmonids were not selective with respect to *Eda* genotype, perhaps because juvenile stickleback do not fully develop their lateral plates until they reach a size larger than those preyed upon in this study. Therefore, ecological context, including features of the predator and prey populations, is critical for drawing inferences about the importance of predators as selective agents on antipredator traits in prey.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

BAW and EPP conceived the study. BAW collected live stickleback samples, analyzed data and wrote the first draft. AKO, CHK, JDK collected salmonids and predated stickleback samples. BAW, KR and OA collected genetic data. All authors contributed to editing the manuscript and approved final submission.

DATA AVAILABILITY STATEMENT

The datasets generated during this study are available on Dryad at <https://doi.org/10.7291/D1MM4X>, (Wasserman et al., 2021).

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