Amino acid cues emanating from Pacific salmon eggs and ovarian fluid

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Abstract

The eggs of salmonid fishes are an important food source for many aquatic predators that detect eggs using olfaction. Moreover, chemicals from eggs and ovarian fluid aid sperm cells in detecting and locating eggs for fertilization, and ovarian fluid is attractive to conspecific males. Thus chemicals from eggs and ovarian fluid may facilitate reproduction but may also attract egg predators. We sampled mature females of three Pacific salmon species: Chinook (*Oncorhynchus tshawytscha*), coho, (*O. kisutch*), and sockeye (*O. nerka*), and determined the proportional representation of amino acids, potent fish odorants, from their eggs and ovarian fluid (Chinook and coho salmon only). We then tested juvenile coho salmon, an egg predator, for responses to ovarian fluid and egg odors using the electro-olfactogram (EOG) recording technique. The amino acid compositions of the salmon species were significantly and positively correlated with each other, and the inter-specific differences were comparable to those between individuals of the same species. The egg water samples were, on average, dominated by lysine, alanine, and glutamine (12.6%, 12.4%, and 10.9%, respectively). The ovarian fluid samples were dominated by lysine (20.5%), followed by threonine (9.7%), glycine (9.2%), and arginine (8.8%). EOG recordings demonstrated the ability of juvenile coho salmon to detect the chemical traces of eggs and ovarian fluid. We conclude that salmon eggs are a potent source of odors for potential predators but likely not highly differentiated among salmon species.

Keywords: attraction, fertilization, odors, ovarian fluid, predation, salmon eggs

Introduction

Salmonid eggs and newly-fertilized embryos are readily consumed by other fishes and may constitute a critical food source despite their limited seasonal availability (Armstrong *et al.,* 2010; Denton *et al*., 2009; Jaecks & Quinn, 2014; Moore *et al.,* 2008; Rinella *et al.,* 2012). Chemical traces of salmonid eggs are highly attractive to predatory salmonid and cottid fishes (Dittman *et al*., 1998; Mirza & Chivers, 2002; Quinn *et al*., 2012). The spawning behavior of salmonids involves egg burial by the female immediately after fertilization (Esteve, 2005), so few eggs are likely to be available to predators very soon after spawning (Greeley, 1932). However, subsequent digging by other females at the same nest site can excavate eggs, releasing them into the water column, so rapid detection and acquisition of eggs benefits predatory fishes.

The release of odorant chemicals from eggs that predators can detect would seem to be maladaptive, so it is likely that they play some other role, either as a byproduct of normal egg development (Finn & Fyhn, 2010) or to attract sperm to facilitate fertilization (Himes *et al.,* 2011; Riffell *et al*., 2002), and perhaps also to avoid hybridization by heterospecifics (Riffell et al., 2004). In salmonids, evidence indicates that ovarian fluid is attractive to mature males (Emanuel & Dodson, 1979) and their activated sperm (Lehnert *et al.*, 2017), and that chemicals from ovarian fluid rather than the eggs stimulate sperm (Rosengrave *et al*., 2009) and mediate against hybridization (Yeates *et al*., 2013), suggesting that the chemical composition of eggs and ovarian fluids differ.

Given the apparent capacity for eggs to produce odors, and the multiple possible roles that they might play including location of breeding sites by adults (Foster, 1985), there is a need to characterize the chemical profiles of eggs and ovarian fluid from different salmon species. In particular, L-amino acids are potent odor sources associated with prey items that can be detected by olfaction (Hara, 2006) and gustation (Yamashita *et al*., 2006) but the extent to which they differ among salmon species has not been investigated. Thus, the objectives of this study were to characterize the amino acid profiles of eggs and ovarian fluid from different Pacific salmon species, and to use electrophysiological techniques to assess the olfactory sensitivity of juvenile salmon to these odors.

Materials and Methods

Collection of egg odors and ovarian fluid

Sockeye salmon (*Oncorhynchus nerka*) eggs were obtained from two mature females that stranded and had just died at the mouth of Hansen Creek, in southwestern Alaska. The body cavities were opened and ovulated eggs were poured through a plastic colander to drain the ovarian fluid and transported to the lab in Ziploc bags. Nitrile gloves were used to handle the fish to prevent contamination with amino acids or other odors from human skin. Local well water was chilled to ~4° C and 250 g of eggs from each female were placed in 1000 ml of water and allowed to soak in a refrigerator with gentle agitation every 5-10 min. After 1 h, the "egg odor water" was decanted into four 250 ml bottles acid-washed and pre-rinsed with deionized milliQ water and immediately frozen (-20° C) to minimize any potential bacterial degradation of odors. Well water controls were similarly collected and frozen.

Eggs were also obtained from one ovulating Chinook salmon (*O. tshawytscha*) and two coho salmon (*O. kisutch*) at the University of Washington salmon hatchery. Nitrile gloves were again used to handle fish to prevent contamination with amino acids from human skin. Females were euthanized by hatchery personnel, the body cavity was opened, and the eggs were poured into a plastic colander to drain the ovarian fluid. Ovarian fluid and eggs were separately collected in Ziploc bags and then transported to the lab. Procedures for making egg odor and control water were the same as for sockeye salmon except that eggs were soaked in chilled (4° C) deionized MilliQ water instead of well water.

Electrophysiological responses to egg odors and ovarian fluid

We examined the olfactory stimulatory effectiveness of the Chinook salmon egg odor water and ovarian fluid, using the electro-olfactogram (EOG) recording technique. EOG responses reflect the summated receptor generator potentials in the olfactory epithelium (Baldwin & Scholz, 2005). Briefly, 1 year-old coho salmon (fork length 15-20 mm) , a salmon egg predator (Armstrong *et al.,* 2010; Quinn *et al.,* 2012) were obtained from the NOAA Northwest Fisheries Science Center, Seattle, WA hatchery, anesthetized with MS-222 (50 mg/L buffered to pH 7), paralyzed with gallamine triethiodide (0.3 mg/100 g body weight), placed in a V-shaped Plexiglas holder, and the gills were perfused with chilled (11°C), oxygenated water containing MS-222 (50 mg/L). A recording electrode was placed near the medial surface of lamellae within the olfactory rosette and its position was adjusted to maximize the response to a standard odorant (10^{-5} M L-serine). A reference electrode was placed on the skin dorsal to the rosette. The rosette of the fish was perfused with artificial freshwater (AFW) (3.0 mM NaCl, 0.2 mM KCl, 0.2 mM $CaCl_2$, 0.4 mM $MgCl_2$, 1.0 mM Hepes, pH 7.2) at all times and pulsed with selected odorants dissolved in AFW chilled to 11°C. Odorants were presented to the epithelium using a computer-activated multi-channel pinch-valve manifold AutoMate (WPI Inc.) that delivered odorants for a 10 s duration. The epithelium was allowed to recover for at least 2 min between stimuli. The odors were delivered in sequence starting with L-serine (10^{-5} M), followed by the test odor, delivered in a series of discrete 10-fold increases from 1:10,000,000 to 1:100, followed by L-serine again for reference. Each concentration of each odor was tested at least twice on each of 5 fish. Odorant-induced neural signals were acquired and filtered with an AC/DC amplifier (A-M Systems Inc.® Model 3000) using Labview software (National Instruments). The magnitude of EOG responses was measured from the baseline prior to stimulation to the peak during the 10 s stimulation. Response to each egg odor concentration was expressed as a percentage of the response to the L-serine (10^{-5} M) standard and then averaged. EOG data were analyzed by ANOVA followed by Tukey's multiple comparison tests (Prism, Graphpad, Inc.)

Amino acid analysis of egg odors and ovarian fluid

Dissolved free amino acid analysis (L-form) was conducted by the Protein Structure Core Facility at University of Nebraska on a Hitachi L-8800A analyzer. Each sample was tested in duplicate except Chinook and coho ovarian fluid. Samples were first reduced in volume using a lyophilizer, then filtered through a 3000 Da MW filter (Pall Inc.) and the flow-through evaporated to dryness. Samples were then dissolved in 100 µl of 0.02 N HCl and 50 µl was injected onto the Amino Acid Analyzer. Norleucine was used as an internal standard to correct for dilution errors. Data analyses were conducted on the percentage values for each amino acid rather than the absolute concentrations because the dilution of the water rendered those arbitrary, and the goal was to assess the extent of similarity in amino acid composition, not the absolute amounts released from the eggs. All amino acids except L-serine were undetectable in the background well water used for creating the sockeye salmon egg solution. The L-serine in this water was detected at levels equivalent to 0.0008% of the egg solution and therefore all subsequent analysis assumed that eggs contributed all the amino acids to the egg solution. The MilliQ purification system should eliminate any amino acid contamination and, therefore, deionized MilliQ water used as background for Chinook and coho salmon egg solutions was not tested for amino acids.

Ethical Statement

All studies were conducted under the guidelines established by the Institutional Animal Care and Use Committee of the University of Washington.

Results

Electrophysiological responses to egg odors and ovarian fluid

Both egg odors and ovarian fluid from Chinook salmon were potent olfactory stimulants of the salmon olfactory epithelium. Ovarian fluid diluted 1:100 elicited a 5.55 ± 0.43 (S.E.M) fold higher response than the single amino acid L-serine tested at 10^{-5} M, a relatively high concentration, and the response to egg odors at 1:100 concentration response was similar to that of the serine (0.92-fold ± 0.16 (S.E.M); Figure 1). As measured by EOG, the threshold for activation of the olfactory epithelium was a 1:1000 dilution of egg odor water and ovarian fluid diluted 1:10000 (Figure 1).

Characterization of egg amino acid profiles among and within species

Pearson correlation coefficients indicated very high correspondence ($P < 0.001$) between the relative concentrations of the 17 L-amino acids from the replicates for the two different egg lots of sockeye (0.997 and 0.994), coho (0.992 and 0.996), and Chinook salmon (0.982) (Table 1). In most cases, the total amino acid concentrations in ovarian fluid and egg odor water were also very similar, however, one sockeye salmon egg solution had over a 10-fold higher concentration than other egg odor water samples. This difference may reflect subtle differences in egg development or collection but percentage molar compositions were similar in all samples. For subsequent analyses of these samples, we used the average percent molar composition of the replicates. The correlations between the ovarian fluid and the egg odor water were positive and significant for coho salmon ($R = 0.642$, $P = 0.003$), and weaker but also significant for Chinook salmon ($R = 0.454$, $P = 0.033$). Correlations between the egg odor water samples from the two coho salmon, and the single sockeye and Chinook salmon, and all were significantly positive (Table 2). However, the correlations within species ($n = 2$, mean = 0.79) did not differ from those between species ($n = 8$, mean = 0.75, $t = 0.43$, P (1-tailed) = 0.34).

Discussion

The salient findings from this study were 1) eggs and ovarian fluid contain and release a variety of amino acids, 2) both sources were potent odors for salmon, based on EOG responses, and 3) the amino acid profiles of each species were significantly and positively correlated with each other, but the variation in profiles from two females within species was comparable to that observed between species. Given the intensive predation by fishes on salmonid eggs, the release of such potent olfactory stimuli from eggs would seem to be maladaptive, as predators could detect the eggs before final visual detection led to consumption. Amino acids may, therefore, function as kairomones (chemicals whose release from one species benefits another species to the detriment of the emitter), and perhaps their release is a requisite byproduct of egg development and physiology (Finn & Fyhn, 2010; Nocillado *et al*., 2000). Amino acids in ovarian fluids may similarly be necessary for successful ovulation, spawning, and fertilization (Finn & Fyhn, 2010; Valdebenito *et al.*, 2010).

High levels of amino acids in salmon eggs were reported by Srivastava *et al.* (1995), but the elevated levels released from intact eggs in the present study were surprising, given the potential for predator attraction. One explanation is that amino acids released from eggs may act as sperm chemo-attractants to facilitate successful external fertilization. Sperm chemo-attractants released from eggs, including amino acids and small peptides (Riffell *et al*., 2002; Yanagimachi *et al*., 2017), have been described for a number of aquatic, broadcast spawning species (Kholodnyy *et al.,* 2020). High levels of amino acids have also been reported in salmonid ovarian fluid (Lahnsteiner *et al*., 1995), and may be necessary for fertilization success as amino acids increased the duration of sperm motility in rainbow trout, *O*. *mykiss* (Valdebenito *et al.,* 2010).

The amino acid profiles in ovarian fluid and egg odor solutions were significantly correlated within and between species, and their EOG response profiles were similar. However, despite these similarities, these two odor sources elicit distinct behavioral responses in predators. For example, sculpin species were strongly attracted to egg odors from sockeye salmon but not to their ovarian fluid (Dittman *et al.,* 1998). This may reflect the complexity of the behavioral responses elicited by different individual amino acids and mixtures. The egg water samples were dominated by three amino acids; lysine, alanine, and glutamine, averaged among all samples, constituted 12.6%, 12.4%, and 10.9%. The ovarian fluid samples were dominated by lysine (20.5%), followed by threonine (9.7%), glycine (9.2%), and arginine (8.8%). Behavioral experiments with juvenile Atlantic salmon, *Salmo salar*, revealed varying reactions to different amino acids, including attraction, repulsion, and no response. Of the three amino acids that dominated the egg odors, the juvenile salmon showed weak positive responses to lysine and alanine, and strong attraction of glutamine (Shparkovskiy *et al*., 1983). Different mixtures of amino acids have been shown to elicit feeding responses (Hara, 2006), avoidance behavior (Rehnberg *et al*., 1985), and act as attractive migratory cues (Yamamoto *et al*., 2013).

Chemicals emanating from eggs and ovarian fluid also have distinct, functional roles as reproductive pheromones and in sperm activation, motility, and attraction (Kholodnyy *et al*., 2020; Zadmajid *et al*., 2019). Maturing male salmon are attracted to the ovarian fluid of females (Emanuel & Dodson, 1979), and can distinguish pre-ovulatory from ovulatory females by chemical cues (Honda, 1980, 1982). Ovarian fluid released during spawning seems to play a primary role in regulating sperm function, including modulating motility, longevity, and velocity (Butts *et al.,* 2012; Rosengrave *et al.,* 2009a; Yeates *et al.*, 2013) whereas chemicals from eggs may be mostly involved in sperm attraction (Kholodnyy et al. 2020; but see Yeates et al. 2013). Despite the correlation of amino acid composition of ovarian fluids between and within species that we observed, species-specific (Yeates et al. 2013), population-specific (Beirao et al., 2015), and individual-specific (Rosengrave et al., 2009) effects of ovarian fluid on sperm function have been described. This suggests that subtle differences in the chemical profiles of an individual's ovarian fluid may elicit distinct functional responses in sperm. It is important to note, however, that these are very complex solutions containing many chemicals other than amino acids, that likely contribute to their olfactory and functional properties (Kholodnyy et al. 2020; Zamaiid et al. 2019).

Finally, we note several important caveats in interpreting olfactory and behavioral responses to the ovarian fluid and egg odors in this study. In a natural setting, egg and ovarian fluid release is behaviorally regulated by individual females as part of their spawning process into very complex hydrological environments (fast-flowing streams and rivers, lakes, etc.). For our experiments, we generated solutions by artificially spawning ovulated females and soaking arbitrary numbers of eggs per liter of water for an arbitrary period of time. Furthermore, there could have been a small, residual amount of ovarian fluid on the eggs used to create egg solutions. Therefore, the relative olfactory sensitivity to these artificial solutions may not reflect the actual concentrations of amino acids that salmon would experience in a natural setting. In addition, the egg solution was produced using water that was not a natural source (i.e., from a well or filtered) and the concentrations of different amino acids may differ from a natural setting where amino acids from other sources and microbial degradation may influence the odors in the water near eggs. For example, any of these factors may have contributed to differences in the total concentration of amino acids we observed between the two sockeye salmon egg samples.

Notwithstanding these caveats, it seems clear that ovulated unfertilized eggs release, and ovarian fluid contains, high levels of amino acids, and the percent molar composition of amino acids from these sources are similar yet distinct. The specific functional role of amino acids in ovarian fluid and eggs remain unclear. Amino acids may be important for embryo development, but the role that their release from eggs plays in fertilization must be balanced with their detection by predators.

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Contributions

A. H. D. and T. P. Q. both contributed to the ideas, data generation, data analysis, manuscript preparation and funding.

Significance Statement

Salmon eggs are an important food source for many aquatic predators that detect eggs using olfaction. Chemicals from eggs and ovarian fluid are important for successful reproduction but may also act as odorant cues for predators. We examined the amino acid profile from eggs and ovarian fluid of Pacific salmon and found the profiles of different salmon species were positively correlated with each other and readily detected by egg predators using olfaction.

References

Armstrong, J. B., Schindler, D. E., Omori, K. L., Ruff, C. P., & Quinn, T. P. (2010). Thermal heterogeneity mediates the effects of pulsed subsidies across a landscape. *Ecology* **91**, 1445-1454

Baldwin, D. H., & Scholz, N. L. (2005). The electro-olfactogram: An in vivo measure of peripheral olfactory function and sublethal neurotoxicity in fish. In G. K. Ostrander (Ed.), *Techniques in Aquatic Toxicology* (pp. 257-276). Boca Raton, FL: CRC Press.

Beirao, J., Purchase, C. F., Wringe, B. F., & Fleming, I. A. (2015). Inter-population ovarian fluid variation differentially modulates sperm motility in Atlantic cod *Gadus morhua*. *Journal of Fish Biology* **87**, 54-68

Butts, I. A. E., Johnson, K., Wilson, C. C., & Pitcher, T E. (2012). Ovarian fluid enhances sperm velocity based on relatedness in lake trout, *Salvelinus namaycush*. *Theriogenology* **78**, 2105-2109

Denton, K. P., Rich, Jr., H. B., & Quinn, T. P. (2009). Diet, movement, and growth of Dolly Varden in response to sockeye salmon subsidies. *Transactions of the American Fisheries Society* **138**, 1207-1219

Dittman, A. H., Brown, G. S., & Foote, C. J. (1998). The role of chemoreception in salmon-egg predation by coastrange (*Cottus aleuticus*) and slimy (*C. cognatus*) sculpins in Iliamna Lake, Alaska. *Canadian Journal of Zoology* **76**, 406-413

Emanuel, M. E., & Dodson, J. J. (1979). Modification of the rheotrophic behavior of male rainbow trout *(Salmo gairdneri)* by ovarian fluid. *Journal of the Fisheries Research Board of Canada* **36**, 63-68

Esteve, M. (2005). Observations of spawning behaviour in Salmoninae: *Salmo, Oncorhynchus*, and *Salvelinus*. *Reviews in Fish Biology and Fisheries* **15**, 1-21

Finn, R. N., & Fyhn, H. J. (2010). Requirement for amino acids in ontogeny of fish. *Aquaculture Research* **41**, 684-716

Foster, N. R. (1985). Lake trout reproductive behavior: influence of chemosensory cues from young-of-the-year by-products. *Transactions of the American Fisheries Society* **114**, 794-803.

Greeley, J. R. (1932). The spawning habits of brook, brown and rainbow trout, and the problem of egg predators. *Transactions of the American Fisheries Society* **62**, 239-248

Hara, T. J. (2006). Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *Journal of Fish Biology* **68**, 810-825

Himes, J. E., Riffell, J. A., Zimmer, C. A., & Zimmer, R K. (2011). Sperm chemotaxis as revealed with live and synthetic eggs. *Biological Bulletin* **220**, 1-5

Honda, H. (1980). Female sex pheromone of rainbow trout, *Salmo gairdneri,* involved in courtship behaviour. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 1109-1112

Honda, H. (1982). On the female pheromones and courtship behaviour in the salmonids, *Oncorhynchus masou* and *O. rhodurus*. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 47-49

Jaecks, T., & Quinn, T. P. (2014). Ontogenetic shift to dependence on salmon-derived nutrients in Dolly Varden char from the Iliamna River, Alaska. *Environmental Biology of Fishes* **97**, 1323-1333

Kholodnyy, V., Gadelha, H., Cosson, J. & Boryshpolets, S. (2020). How do freshwater fish sperm find the egg? The physicochemical factors guiding the gamete encounters of externally fertilizing freshwater fish. *Reviews in Aquaculture* **12**, 1165-1192.

Lahnsteiner F., Weismann, T., & Patzner, R. A. (1995). Composition of the ovarian fluid in 4 salmonid species: *Oncorhynchus mykiss, Salmo trutta f lacustris, Salvelinus alpinus* and *Hucho hucho. Reproduction Nutrition Development* **35**, 465-474

Lehnert, S. J., Butts, I. A. E., Flannery, E. W., Peters, K. M., Heath, D. D., & Pitcher, T. E. (2017). Effects of ovarian fluid and genetic differences on sperm performance and fertilization success of alternative reproductive tactics in Chinook salmon. *Journal of Evolutionary Biology* **30**, 1236-1245

Mirza, R. S., & Chivers, D. P. (2002). Attraction of slimy sculpins to chemical cues of brook charr eggs. *Journal of Fish Biology* **61**, 532-539

Moore, J. W., Schindler, D. E., & Ruff, C. P. (2008). Habitat saturation drives thresholds in stream subsidies. *Ecology* **89**, 306-312

Nocillado J. N., Penaflorida, V. D., & Borlongan, I. G. (2000). Measures of egg quality in induced spawns of the Asian sea bass, *Lates calcarifer Bloch. Fish Physiology and Biochemistry* **22**, 1-9

Quinn, T. P., Dittman, A. H., Barrett, H., Cunningham, C., & Bond, M. H. (2012). Chemosensory responses juvenile coho salmon, *Oncorhynchus kisutch*, Dolly Varden, *Salvelinus malma*, and sculpins (*Cottus* spp.) to eggs and other tissues from adult Pacific salmon. *Environmental Biology of Fishes* **95**, 301-307

Rehnberg B. G., B. Jonasson and C. B. Schreck. 1985. Olfactory sensitivity during parr and smolt developmental stages of coho salmon. *Transactions of the American Fisheries Society* **114**, 732-736

Riffell, J. A., Krug, P. J., & Zimmer, R. K. (2002). Fertilization in the sea: the chemical identity of an abalone sperm attractant. *Journal of Experimental Biology* **205**, 1439–1450

Riffell, J. A., Krug, P. J., & Zimmer, R. K. (2004). The ecological and evolutionary consequences of sperm chemoattraction. *Proceedings of the National Academy of Sciences* **101**, 4501–4506

Rinella, D. J., Wipfli, M. S., Stricker, C. A., Heintz, R. A., & Rinella, M J. (2012). Pacific salmon (*Oncorhynchus* spp.) runs and consumer fitness: growth and energy storage in stream-dwelling salmonids increase with salmon spawner density. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 73–84

Rosengrave, P., Gemmell, N. J., Metcalf, V., McBride, K., & Montgomerie, R. (2008). A mechanism for cryptic female choice in Chinook salmon. *Behavioral Ecology* **19**, 1179–1185

Rosengrave, P., Taylor, H., Montgomerie, R., Metcalf, V., McBride, K., & Gemmell, N. J. (2009). Chemical composition of seminal and ovarian fluids of chinook salmon (*Oncorhynchus tshawytscha*) and their effects on sperm motility traits. *Comparative Biochemistry and Physiology A* **152**, 123-129

Shparkovskiy, I. A., Pavlov, I. D., & Chinarina, A. D. 1983. Behavior of young hatchery-reared Atlantic *Salmo salar*, (Salmonidae) influenced by amino acids. *Journal of Ichthyology* **23**, 140-147.

Srivastava R. K., Brown, J. A., & Shahidi, F. (1995). Changes in the amino acid pool during embryonic development of cultured and wild Atlantic salmon (*Salmo salar*). *Aquaculture* **131**, 115-124

Valdebenito I., Moreno, C., Lozano, C., & Ubilla, A. (2010). Effect of L-glutamate and glycine incorporated in activation media, on sperm motility and fertilization rate of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. *Journal of Applied Ichthyology* **26**, 702-706

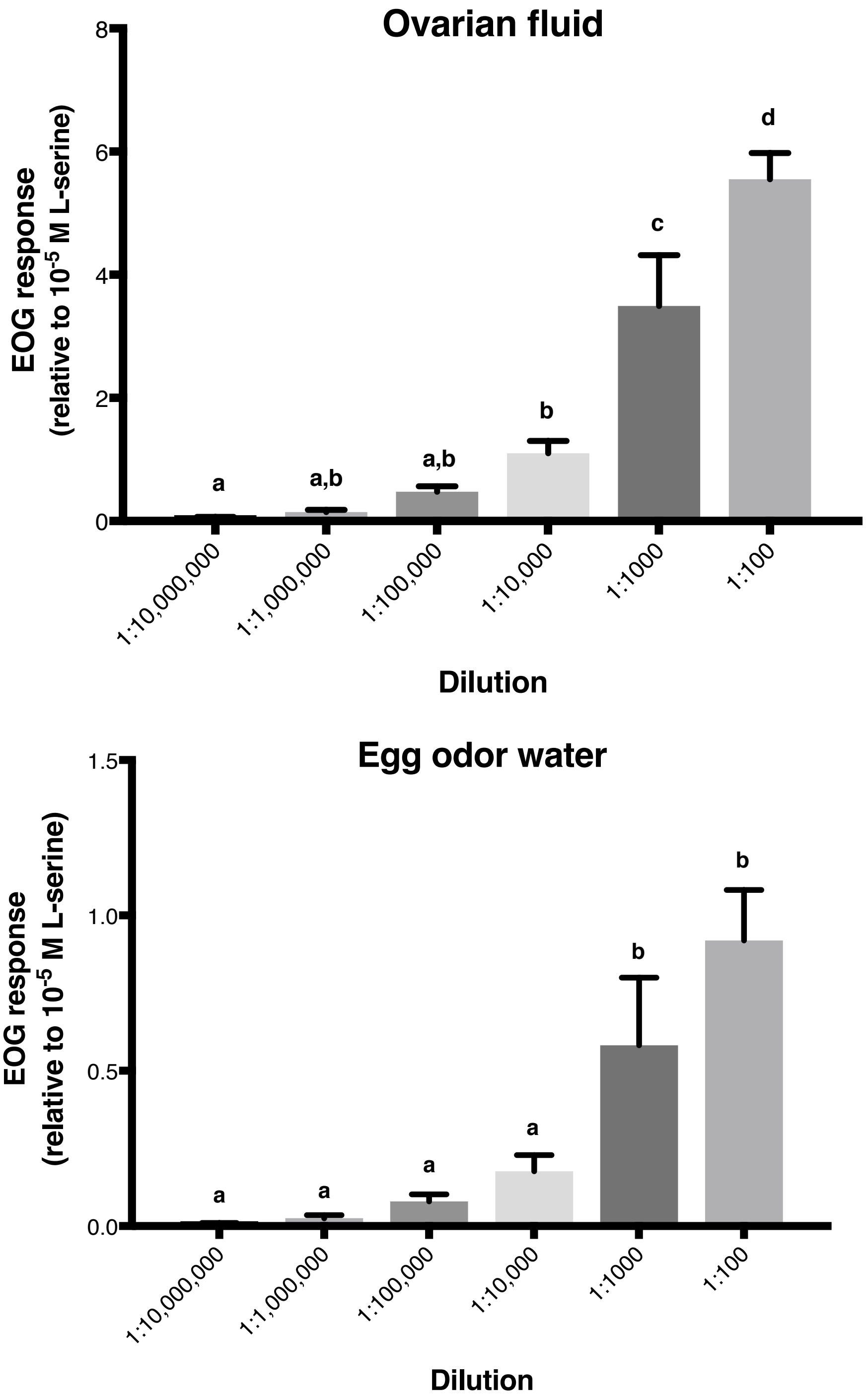
Yamamoto, Y., Shibata, H., & Ueda, H. (2013). Olfactory homing of chum salmon to stable compositions of amino acids in natal stream water. *Zoological Science* **30**, 607-612

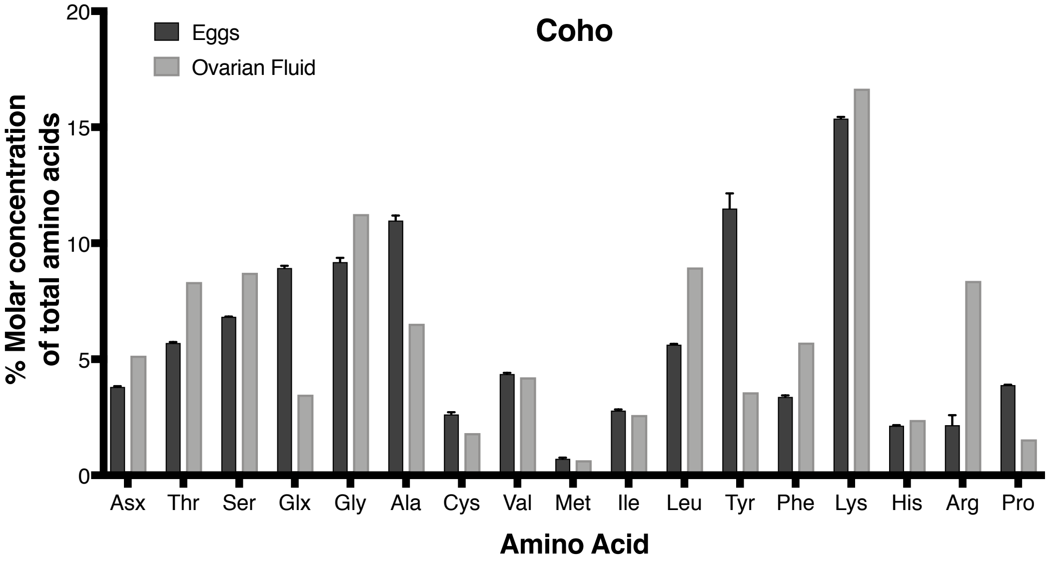
Yamashita, S., Yamada, T., & Hara, T. J. (2006). Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *Journal of Fish Biology* **68**, 783-800

Yanagimachi, R., Harumi, T., Matsubara, H., Yan, W., Yuan, S., Hirohashi, N., Iida, T., Yamaha, E., Arai, K., Matsubara, T., Andoh, T., Vines, C., & Cherr, G. N. (2017). Chemical and physical guidance of fish spermatozoa into the egg through the micropyle. *Biology of Reproduction* **96**, 780–799

Yeates, S. E., Diamond, S. E., Einum, S., Emerson, B. C., Holt, W. V., & Gage, M. J. G. (2013). Cryptic choice of conspecific sperm controlled by the impact of ovarian fluid on sperm swimming behavior. *Evolution* **67**, 3523–3536

Zadmajid, V., Myers, J. N., Sorensen, S. R., & Butts, I. A. E. (2019). Ovarian fluid and its impacts on spermatozoa performance in fish: A review. *Theriogenology* **132**, 144-152


Ovarian fluid
EOG response
(relative to 10^{-5} M L-serine)
0
2
4
6
8
a
a,b
a,b
b
c
d
1:10,000,000
1:1,000,000
1:100,000
1:10,000
1:1000
1:100
Dilution
Egg odor water
EOG response
(relative to 10^{-5} M L-serine)
0.0
0.5
1.0
1.5
a
a
a
a
b
b
1:10,000,000
1:1,000,000
1:100,000
1:10,000
1:1000
1:100
Dilution


Coho
Eggs
Ovarian Fluid
% Molar concentration of total amino acids
0
5
10
15
20
Asx
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Met
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Leu
Tyr
Phe
Lys
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Arg
Pro
Amino Acid

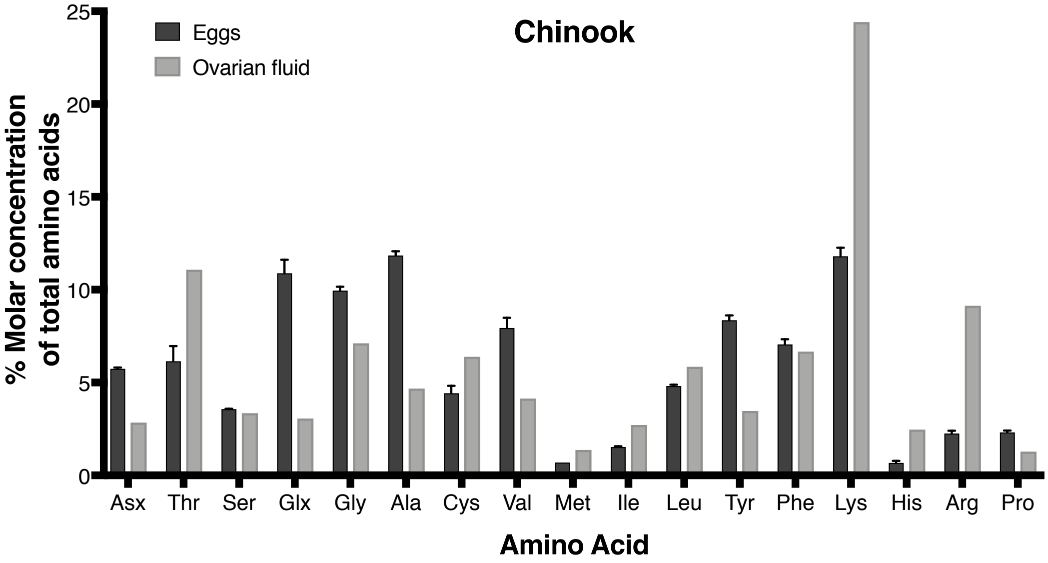
Chinook
Eggs
Ovarian fluid
% Molar concentration of total amino acids
0
5
10
15
20
25
Asx
Thr
Ser
Glx
Gly
Ala
Cys
Val
Met
Ile
Leu
Tyr
Phe
Lys
His
Arg
Pro
Amino Acid

Figure captions

Figure 1. Mean (± SEM) electro-olfactogram (EOG) responses of juvenile coho salmon (*Oncorhynchus kisutch*) to sequential dilutions of ovarian fluid and odors of eggs from Chinook salmon (*O. tshawytscha*), expressed relative to responses to L-serine. EOG responses that do not share lowercase letters are significantly different at p=0.05 level using Tukey's multiple comparison test. Note different scales for EOG response for ovarian fluid and egg odor water.

Figure 2. Percent molar concentrations of different L-amino acids from ovarian fluid and odor of eggs (means ± S.E.M) of Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon.

Salmon eggs are an important food source for many aquatic predators that detect eggs using olfaction. Chemicals from eggs and ovarian fluid are important for successful reproduction but may also act as odorant cues for predators. We examined the amino acid profile from eggs and ovarian fluid of Pacific salmon and found the profiles of different salmon species were positively correlated with each other and readily detected by egg predators using olfaction.

Table 1. Percent molar composition of 17 L-amino acids in water that had contained eggs of one Chinook (*Oncorhynchus tshawytscha*), two coho (*O. kisutch*), and two sockeye salmon (*O. nerka*), and the ovarian fluid (OF) from the Chinook salmon and one of the two coho salmon. The values for the egg odor water represent the averages of two replicate runs of the same samples. Total concentrations are reported at the bottom in nM.

Amino acid	Chinook egg	Chinook OF	Coho 1 egg	Coho 1 OF	Coho 2 egg	Sockeye 1 egg	Sockeye 2 egg
Asparagine	5.7	2.8	3.8	5.1	11.8	5.9	7.4
Threonine	6.2	11.1	5.7	8.3	6.2	10.7	8.1
Serine	3.6	3.4	6.8	8.7	10.1	8.9	8.1
Glutamine	10.9	3.1	8.9	3.5	12.1	11.7	10.9
Glycine	10.0	7.1	9.2	11.3	2.6	7.0	8.6
Alanine	11.8	4.7	11.0	6.5	10.8	14.1	14.1
Cysteine	4.4	6.4	2.6	1.8	1.1	3.3	2.2
Valine	7.9	4.1	4.4	4.2	5.5	6.5	5.1
Methionine	0.7	1.4	0.7	0.6	0.7	0.4	1.0
Isoleucine	1.5	2.7	2.8	2.6	2.4	3.3	2.9
Leucine	4.8	5.8	5.6	9.0	5.9	6.5	6.8
Tyrosine	8.4	3.5	11.5	3.6	4.0	2.1	4.6
Phenylalanine	7.0	6.7	3.4	5.7	2.5	4.9	3.1
Lysine	11.8	24.4	15.4	16.7	14.9	9.9	11.1
Histidine	0.7	2.5	2.1	2.4	4.0	1.1	1.1
Arginine	2.3	9.1	2.2	8.4	4.4	2.5	2.6
Proline	2.3	1.3	3.9	1.5	1.1	1.3	2.2
Total amino acid conc. (nM)	25.48	269.66	73.52	378.17	52.60	30.38	732.03

Table 2. Pearson correlation coefficients between L-amino acid profiles of water in which eggs of two different female coho salmon (*Oncorhynchus kisutch*), one Chinook salmon (*O. tshawytscha*), and two sockeye salmon (*O. nerka*) had been immersed. All values are significant at P < 0.025 in one-tailed tests.

	Coho 1	Coho 2	Sockeye 1	Sockeye 2
Chinook	0.84	0.60	0.76	0.82
Coho 1		0.64	0.64	0.78
Coho 2			0.76	0.82
Sockeye 1				0.94