Preference Alters Consumptive Effects of Predators: Top-Down Effects of a Native Crab on a System of Native and Introduced Prey

Emily W. Grason\textsuperscript{1,2\ast}, Benjamin G. Miner\textsuperscript{1,2}

\textsuperscript{1}Western Washington University, Biology Department, Bellingham, Washington, United States of America, \textsuperscript{2}Shannon Point Marine Center, Anacortes, Washington, United States of America

\textbf{Abstract}

Top-down effects of predators in systems depend on the rate at which predators consume prey, and on predator preferences among available prey. In invaded communities, these parameters might be difficult to predict because ecological relationships are typically evolutionarily novel. We examined feeding rates and preferences of a crab native to the Pacific Northwest, \textit{Cancer productus}, among four prey items: two invasive species of oyster drill (the marine whelks \textit{Urosalpinx cinereai} and \textit{Ocenebra inornata}) and two species of oyster (\textit{Crassostrea gigas} and \textit{Ostrea lurida}) that are also consumed by \textit{U. cinereai} and \textit{O. inornata}. This system is also characterized by intraguild predation because crabs are predators of drills and compete with them for prey (oysters). When only the oysters were offered, crabs did not express a preference and consumed approximately 9 juvenile oysters crab\textsuperscript{-1} day\textsuperscript{-1}. When we tested whether crabs preferred adult drills of either \textit{U. cinereai} or \textit{O. inornata}, or juvenile oysters (\textit{C. gigas}), while consumed drills and oysters at approximately the same rate when only one type of prey was offered, they expressed a strong preference for juvenile oysters over drills when they were allowed to choose among the three prey items. This preference for oysters might negate the positive indirect effects that crabs have on oysters by consuming drills (trophic cascade) because crabs have a large negative direct effect on oysters when crabs, oysters, and drills co-occur.

\textbf{Introduction}

Predation is a major force structuring un-invaded [1,2] and invaded [3,4] communities. Directly, predators can limit [5,6], regulate [7], or extirpate [8] prey populations through consumption. These effects can, in turn, be propagated indirectly through the community in many ways, including trophic cascades, indirect facilitation, and apparent competition [9]. Direct and indirect effects of predation can engender quantitative, as well as qualitative, shifts in community composition. For instance, predators can enhance community diversity by consuming competitively dominant prey, thereby reducing interspecific competition and facilitating the persistence of competitively inferior prey [10]. The population and community effects of predators depend on the number of prey they consume, and their preference.

The trophic dynamics of an invaded food web are not always predictable because predator-prey interactions are typically evolutionarily novel. Predators might not be able to recognize an invasive prey as potential food, or overcome defenses of invasive prey [11]. Even where predators are generalists and invasive prey are ecologically similar to natives, an invaded trophic chain might not function in the same way as a comparable chain comprised only of co-evolved natives [12]. Prey preference in the European green crab, \textit{Carcinus maenas}, destabilized competitive interactions between native and non-native cannans, allowing the established non-native gem clam, \textit{Genus gemma}, to become invasive [13]. Appropriate management of invasions therefore requires investigation of the pathways by which predators impact the community.

We investigated a commercially and ecologically important food web with a native predator, mediated by invasive intermediate prey (Figure 1). In Washington State, two invasive species of oyster drill, the marine whelks \textit{Ocenebra inornata} and \textit{Urosalpinx cinerea}, are common predators of oysters. Because drills threaten the recovery efforts of a rare native oyster (\textit{Ostrea lurida}) and yield of commercial oyster harvest, the effects of drills as predators of oysters have been well studied [14–16]. Drills of both species generally prefer to prey on small oysters, and can consume juveniles at a rate of 0.3 oysters per day [16] potentially limiting oyster populations at this life history stage. However, relatively little is known about predatory control that native crabs, which are common predators of marine snails, exert on this system [but see (12)]. As predators of both drills and oysters, crabs could impact oysters directly by preying on
Preference Alters Consumptive Effects of Predators

and preference of crabs differed for the two oyster species. We then investigated crab feeding on both species of invasive drill and non-native *C. gigas*.

**System**

Both Atlantic (*U. cinerea*) and Japanese oyster drills (*O. inornata*, synonyms include *Cerastostoma inornatum*, and *Ocenebrellus inornatus*) were unintentionally introduced to the Pacific Northwest by the early 1920’s [24]. Both drills arrived as hitchhikers on cultch (newly settled larvae on shell material) of non-native oysters imported to buoy the oyster-growing industry – *U. cinerea* from the east coast of the United States on *Crassostrea virginica* (Eastern oyster), and *O. inornata* from the Asian Pacific Ocean on *Crassostrea gigas* (Pacific oyster). Because they lack a larval planktonic stage, drills are dispersed primarily through human-mediated transport. Notably, native whelks are not considered a pest in oyster beds [24], so we did not include them in our study.

While at least five species of oyster are currently grown and harvested in Washington, we were particularly interested in the effects of crabs on non-native *C. gigas* and native *O. lurida*. The Pacific oyster, *C. gigas*, is the most widely introduced, and commercially valuable oyster species worldwide, and has also established naturally recruiting, wild populations in bays and inland waters of Puget Sound and coastal Washington [25]. In contrast, populations of *O. lurida*, the only native oyster species in Washington, collapsed in the late 1800’s due to overharvesting and pollution, and have not sustained a harvestable wild population since [26]. Drills are pests to the oyster industry, costing shellfish growers money in control and eradication efforts, as well as in decreased revenues. Additionally, drills could be inhibiting recovery efforts aimed at restoring wild populations of the rare and ecologically important native *O. lurida* [16,27].
Due to their relatively thin shells, juveniles of both oyster species are most vulnerable to drill predation [16]. Both drills consume small individuals of C. gigas and O. lurida at similar rates of about 0.25–0.30 oysters per drill per day [16]. Preference for the two species of drills between these two species of oyster has already been established and so was not tested here. Despite divergent evolutionary histories with these oyster prey, both drills prefer C. gigas to O. lurida oysters of similar size [16]. Population growth of oysters depends on at least some recruits reaching reproductive size without being consumed. Survival of oyster populations, therefore, could be strongly affected by predation on juvenile oysters by adult drills (E. Grason, unpublished data). Buhle and Ruesink [16] hypothesized that crab predation might limit distribution of drills, and therefore their effects on oysters, but there is, as yet, no experimental support for this hypothesis.

Animal Collection and Husbandry
Both male and female red rock crabs, C. productus, were collected by hand and trap from beaches and docks around Anacortes, WA. We assumed crabs had no experience with either species of drill or oyster because those organisms or their remains were not found in the areas where crabs were collected. Neither oysters nor invasive drills were present at these sites, likely because oysters have not historically been cultured at these sites. This reduces the probability that crabs had prior experience with any of the species of prey used in our experiments and allows us to infer that preferences are innate. While in the lab, crabs were maintained in flow-through aquaria, on a diet of mussels (Mytilus sp.) and frozen fish fillet (Trachurus sp.). Crabs were not starved prior to the feeding experiments.

Atlantic drills, U. cinerea, were collected from naturally recruiting C. gigas reefs in the southeastern corner of Willapa Bay, WA. Japanese drills, O. inornata, were collected from commercial C. gigas beds owned by Taylor Shellfish Farms in West Samish Bay, WA. Drill species were maintained in separate, closed 140 L aquaria, and allowed to feed ad libitum on mussels and C. gigas juveniles.

Juvenile oysters of both species were obtained as seed (singles) from Taylor Shellfish Farm hatcheries. At the time of experiments, oysters were of a size at which they could typically be out-planted by commercial or recreational growers (C. gigas = 2.7±0.4 cm, O. lurida = 2.5±0.2 cm). Oysters were held in sea-tables and had access to a limited amount of plankton that came in with the natural seawater. We supplemented this diet with commercial shellfish diet (Shellfish Diet 1800-Reed Mariculture) at least once a week.

Preference Experiments
For the purpose of this study, preference was defined as a deviation of feeding behavior (proportion of prey consumed of one type) in the presence of choice compared to feeding behavior without choice [28]. Therefore each experiment included treatments in which crabs were offered one prey type only, and one treatment where all prey types were offered simultaneously in equal abundance. This design has the advantage of providing researchers with several relevant estimates of feeding rate and clearly differentiates between preference and electivity (for a discussion of “preference” versus “electivity” see [29]). Electivity can cause diet changes in a predator via “prey switching”, which occurs when predators attack different prey depending on relative prey abundance [30]. Such “switching” behavior can be ecologically relevant, but was not tested in this experiment, as we chose to focus on preference as a trait of crabs, rather than how contexts affect the interaction between crabs and the two types of prey.

Two experiments were conducted to estimate preference and feeding rates among the prey items. We first estimated C. productus feeding rates on and preferences among juvenile oysters, Pacific (C. gigas) and Olympia (O. lurida) oysters (September, 2009). Then, we estimated C. productus feeding rates on and preferences for juvenile oysters, (C. gigas) and both species of drill (U. cinerea and O. inornata) (October, 2009). Conducting these experiments separately, rather than as a single experiment with all four prey types, allowed for greater replication. In this way we improved resolution in determining crab preference between the two species of oyster because we thought that a general preference for oysters over drills might obscure any difference between the two oyster species. Comparing preference among adult drills and juvenile oysters was ecologically relevant to oyster restoration efforts as well as aquaculture scenarios. Oysters in both experiments were marked with enamel to facilitate correct species identification. Different individual crabs were used in each of the experiments. In the first experiment, crabs (mean carapace width ± SE: 106.7±2.1 mm) were randomly assigned to one of three treatments (n = 12): (1) 25 C. gigas only, (2) 25 O. lurida only, or (3) 25 C. gigas and 25 O. lurida. In the second experiment, crabs (mean carapace width ± SE: 107.2±2.2 mm) were randomly assigned to one of four treatments (n = 14): (1) 25 C. gigas only, (2) 25 O. inornata only, (3) 25 U. cinerea only, or (4) 25 C. gigas, 25 O. inornata, and 25 U. cinerea. We used only C. gigas in this experiment because there was no preference between oyster species in our first experiment and this species is more readily available and not of conservation concern. Observation confirmed that there was no predation by drills on oysters during this experiment, and all oyster mortality was due to crab predation.

One day prior to each experiment, individual crabs were placed in separate flow-through bins with 10 individuals of their assigned prey types to allow feeding behavior to stabilize [31]. At the start of the experiment, we removed all waste, uneaten food, and shell material from the bins, and added individuals of each prey type appropriate for the treatment of each bin. The number of surviving prey of each type was recorded at 24 hours without replacement. In one replicate, a crab consumed all available prey of one type (all individuals of C. gigas consumed by one crab in choice treatment of oyster preference experiment).

Analysis
To determine whether crabs preferred one type of prey, we developed a new method, which is described below. Currently there is debate about how best to statistically test for preference, and the proposed methods all have benefits and drawbacks [28,32–35]. With all available methods, it is difficult to test for preference with more than two species. Because we wanted to test for preference for more than two prey species, we developed an alternative method carefully considering the concerns raised in previous studies [26,30–33].

To test for preference, we used the interaction term of a two-factor ANOVA with prey type and choice both modeled as fixed factors. Prey type had two levels in the first experiment (two species of oyster) and three levels in the second experiment (two species of drill and one species of oyster), and choice had two levels in both experiments (whether crabs had a choice of prey or not). The response variable, the proportion of prey consumed, was calculated as follows. In the no-choice bins, we randomly grouped one replicate from each prey type together and calculated the proportion of prey consumed for each prey type out of the total prey consumed for the group. In the choice treatment, we calculated the proportion of prey consumed for each prey type out of the total prey consumed in a single replicate bin. If the
proportion of each prey type consumed was different when crabs were offered a choice, versus when crabs were only offered a single type, the interaction term of a two-factor ANOVA with prey type and choice as fixed factors would be significant. However, because of our calculations of the response variable, the data were not independent—the proportions of each prey type consumed in each replicate where crabs were allowed a choice were, by definition, constrained to total to 1.0. Therefore, use of the F-statistic distribution would calculate an incorrect probability of type I error. We therefore generated a null distribution of F-ratios for the interaction between choice and prey type by randomly assigning prey types to bins in both the no choice and choice treatments for each experiment (10,000 iterations). We used the generated null distribution to calculate the probability of a type I error for the observed F-ratio for the interaction between choice and prey type.

To ensure that the results were not particular to a random pairing of bins in the no choice treatment, we randomly re-paired bins in the no choice treatment and re-ran the analysis 1,000 times. Distributions of P values of the interaction between choice and prey type were generated and compared to an alpha value of 0.05 to determine whether crabs preferentially fed on the prey species. All analyses were conducted in R [36].

Results

Oyster Preference Experiment

There was no evidence that crabs preferred one species of juvenile oyster to the other (Figure 2). Crabs consumed a similar number of C. gigas as juvenile O. lurida when they were offered only one species and denied a choice, but consumed slightly more C. gigas than O. lurida when offered a choice. However, all of the P values of the interaction between prey type and choice generated in the random pairing of bins were greater than 0.05, suggesting that the interaction term is not significant. Crabs, therefore, do not consume a different proportion of juvenile C. gigas and O. lurida in the presence and absence of choice.

Pacific Oysters and Drills Preference Experiment

There was evidence that crabs preferred non-native oysters to drills (Figure 3). When offered only one type of prey, crabs did not consume prey types at different rates. An average of 7 juvenile C. gigas, 7 O. lurida, or 9 U. cinerea were consumed per crab per day in the absence of choice. However, in treatments in which crabs were allowed to choose from among the three prey types, oysters were disproportionately preyed on compared to drills—crabs consumed nearly 6 times as many oysters as either species of drill. Approximately 99.9% of the P values of the interaction between factors prey species and choice generated by randomly pairing bins were less than 0.05, indicating that crabs consumed a significantly different proportion of oysters in the presence and absence of choice.

Discussion

Whether or not crabs demonstrated a preference among prey choices depended on the types of prey offered. In the first experiment, when both prey were oysters, the predator expressed no preference for either the native or the invasive oyster. In the second experiment, when both species of drill were offered along with one species of oyster, crabs strongly preferred C. gigas, oysters, to either species of drill. This difference is likely not a product of different handling times for each prey species, as all prey types were consumed at relatively similar rates when offered in single species treatments. It is probable that the preference for C. gigas

Figure 2. Crab feeding rates and preference on native and non-native oysters. Number (mean ± SE) of oysters consumed by crabs over 24 hours in the oyster preference experiment. Gray bars are Crassostrea gigas, Pacific oysters, and white bars are Ostrea lurida, Olympia oysters. Crabs were randomly assigned to one of three treatments (n = 12): (1) 25 C. gigas only, (2) 25 O. lurida only, or (3) the treatment labeled “Choice”, 25 C. gigas and 25 O. lurida. doi:10.1371/journal.pone.0051322.g002

results from a relatively greater energy yield per unit effort required to obtain the food from oysters as opposed to well-armored drills.

At sites where crabs, drills, and oysters co-occur, crab preference could cause a direct negative effect on juvenile oysters that negates the positive indirect effect of crabs eating drills. In our treatment that allowed crabs to choose, crabs consumed an average of about 6 oysters and 2 drills (one of each species) per day. Individual drills consume at most approximately 0.3 juvenile oysters per day [16]. In the presence of choice, therefore, the direct negative effect of an individual crab on oysters is approximately -6 oysters per day, while the positive indirect effect is +0.6 oysters per day. The net effect of crabs in the system is still strongly negative: 5.4 oysters removed per crab per day. Therefore, despite the fact that they can be highly efficient and motivated predators on drills, C. productus is likely to exert stronger direct consumptive effects on oysters. Long-term dynamics in this system will depend on population responses of oysters to predation by both crabs and drills. For instance, predation on oysters could facilitate a population increase in crabs that could then impact drills negatively via apparent competition, or deplete oysters to the extent that crabs switch their search image to prey primarily on drills.

As an intraguild predator, therefore, C. productus interacts more strongly with drills as a competitor for oysters than as a predator. It is notable that this crab-drill-oyster system does not have the characteristics of systems in which IGP is believed to be stabilizing. In asymmetric IGP systems, a condition for stability is that the intraguild predator is the stronger competitor for the extraguild resource [37]. In this study, not only is crab-drill predation unidirectional, but it also seems likely, based on differences in per
capita feeding rates, that crabs are better at exploiting oysters than drills. Other researchers have pointed out that models of IGP stability have required systems of closed populations, a condition which is clearly not met in many marine habitats where species often have widely-dispersing pelagic larvae [21]. Thus the population growth rate of oysters in our system might be too low, even when oysters are present. Additionally, along with emigration and predation, inducible defenses of drills in response to crabs likely carry fitness costs that explain lower drill densities where C. produtus is present.

Our study suggests the interesting possibility that oysters facilitated the invasion of both species of drill, not only as a vector (non-native oysters) and food source (both native and non-native oysters), but also by reducing the potential for biotic resistance by native crabs. Both drills were originally introduced simultaneously with oysters, and, notably, are almost entirely restricted to oyster beds in Washington State. This granted drills a degree of enemy release, at least in the short term, because crabs preferentially prey on oysters when they have a choice. The corollary to this idea is that while C. produtus might not strongly affect drill populations in oyster beds, crabs could help limit the range of invasive drills to oyster beds. Where oysters are rare, it is possible that crabs will switch to consuming relatively more drills, and crabs could thereby provide greater biotic resistance against drill incursion into these habitats. This provides one way that context-dependent species interactions, such as those mediated by preference, could be particularly important in invaded systems.

Acknowledgments

We are very grateful for logistical support for this research contributed by Taylor Shellfish Farms and the Pacific Northwest Shell Club. Helpful feedback on manuscripts was provided by B. Bingham and M. Peterson.

Author Contributions

Conceived and designed the experiments: EG BM. Performed the experiments: EG. Analyzed the data: BM. Contributed reagents/materials/analysis tools: EG. Wrote the paper: EG.

References

1. Paine RT (1974) Intertidal community structure - experimental studies on relationship between a dominant competitor and its principal predator. Oecologia 13: 93–120.
2. Hixon MA, Been JP (1995) Predation, prey refuges, and the structure of coral-reef fish assemblages. Ecological Monographs 65: 77–101.
3. Sato P, Korpimaki E, Banks PB, Nordstrom M, Dickman CR (2007) Alien predators are more dangerous than native predators to prey populations. Proceedings of the Royal Society B-Biological Sciences 274: 1237–1243.
4. Gheltzard F, Acquapare P (2007) Invasive crayfish in Europe: the impact of Procambarus clarkii on the littoral community of a Mediterranean lake. Freshwater Biology 52: 1249–1259.
5. Finzner RE, Eberhart LE, Rickard WH, Gray RH (1994) Great Basin Canada goose nesting in the mid-Columbia River, Washington: An historical perspective and update, 1981–1990. Northwest Science 68: 37–42.
6. Giereich J, Morrison JA, Hodges LV (2000) The interaction between competition and predation: a meta-analysis of field experiments. American Naturalist 155: 435–453.
7. Korpimaki E, Krebs CJ (1996) Predation and population cycles of small mammals – A reassessment of the predation hypothesis. Bioscience 46: 759–764.
8. Kavanagh RP (1986) The impact of predation by the Powerful owl, Ninox strenae, on a population of the Greater glider, Petauroides volans. Australian Journal of Ecology 13: 435–450.
9. Wootten JT (1994) The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25: 443–466.
10. Paine RT (1966) Food web complexity and species diversity. The American Naturalist 100: 65–75.
11. Carlson NO, Sarnelle O, Strayer DL (2009) Native predators and exotic prey - an acquired taste? Frontiers in Ecology and the Environment 7: 525–532.
12. Kimbro DL, Grosholz ED, Baskus AJ, Nesbitt NJ, Travis NM, et al. (2009) Invasive species cause large-scale loss of native California oyster habitat by disrupting trophic cascades. Oecologia 160: 568–575.

13. Grosholz ED (2005) Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. PNAS 102: 1092–1097.

14. Hanks JE (1957) The rate of feeding of the common oyster drill, Urosalpinx cinerea (Say), at controlled water temperatures. Biological Bulletin 112: 330–335.

15. Chew KK (1960) Study of food preference and rate of feeding of Japanese Oyster Drill, Ostrea japonica (Dunker). Washington, D.C.: United States Fish and Wildlife Service.

16. Buhrle ER, Ruesink JL (2009) Impacts of invasive oyster drills on Olympia oyster (Ostrea lurida Carpenter 1864) recovery in Willapa Bay, Washington, United States. Journal of Shellfish Research 28: 87–96.

17. Holman KK, McDonald PS, Armstrong DA (2006) Intertidal migration and habitat use by subadult Dungeness crab Cancer magister in a NE Pacific estuary. Marine Ecology Progress Series 308: 103–105.

18. Yamada SB, Boulding EG (1996) The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. Journal of Experimental Marine Biology and Ecology 204: 55–63.

19. Rohr G, Myers C, Hob R (1989) The Ecology and Evolution of Intraguild Predation - Potential Competitors That Eat Each Other. Annual Review of Ecology and Systematics 20: 297–330.

20. Menge B, Lubchenco J, Gaines S, Ashkenas L (1986) A Test of the Menge-Sutherland Model of Community Organization in a Tropical Rocky Intertidal Food Web. Oecologia 67: 75–89.

21. Navarrete SA, Menge BA, Daley BA (2000) Species interactions in intertidal food webs: Prey or predation regulation of intermediate predators? Ecology 81: 2264–2277.

22. Conchamch F, Langhais M, Sugihara G (1999) Cues protecting birds: modelling the masquerader release effect. Journal of Animal Ecology 68: 282–292.

23. Hall RJ (2011) Eating the competition speeds up invasions. Biology Letters 7: 307–311.

24. Chapman WM, Banner AH (1949) Contributions to the life history of the Japanese oyster drill, Tritonella japonica (= Cerastoma inermatum), with notes on other enemies of the Olympia oyster, Ostrea lurida. Washington Department of Fisheries.

25. Ruesink JL, Leitahan HS, Trumble AC, Heiman KW, Micheli F, et al. (2005) Introduction of non-native oysters: Ecosystem effects and restoration implications. Annual Review of Ecology Evolution and Systematics 36: 643–669.

26. White J, Ruesink JL, Trumble AC (2009) The nearly forgotten oyster: Ostrea lurida Carpenter 1864 (Olympia Oyster) history and management in Washington State. Journal of Shellfish Research 28: 43–49.

27. Cook AE, Shaffer JA, Dumbauld BR, Kauffman BE (2000) A plan for rebuilding stocks of Olympia oysters (Ostrea lurida, Carpenter 1857) in Washington State. Journal of Shellfish Research 19: 409–412.

28. Underwood AJ, Chapman MG, Gower TP (2004) Identifying and understanding ecological preferences for habitat or prey. Journal of Experimental Marine Biology and Ecology 300: 161–187.

29. singer MG (2000) Reducing ambiguity in describing plant-insect interactions: “preference”, “acceptability” and “eating.” Ecology Letters 3: 159–162.

30. Murdoch W (1969) Switching in General Predators. Experiments on Predator Specificity and Stability of Prey Populations. Ecological Monographs 39: 335–354.

31. Jackson AG, Underwood AJ (2007) Application of new techniques for the accurate analysis of choice of prey. Journal of Experimental Marine Biology and Ecology 341: 1–9.

32. Maina BJF (1993) Comments on design and analysis of multiple-choice feeding-preference experiments. Oecologia 93: 149–152.

33. Underwood AJ, Clarke KR (2000) Solving some statistical problems in analyses of experiments on choices of food and on associations with habitat. Journal of Experimental Marine Biology and Ecology 308: 227–237.

34. Taplin RH (2007) Experimental design and analysis to investigate predator preferences for prey. Journal of Experimental Marine Biology and Ecology 344: 116–122.

35. Underwood AJ, Clarke KR (2007) More response on a proposed method for analysing experiments on food choice. Journal of Experimental Marine Biology and Ecology 344: 113–115.

36. R Development Core Team (2009) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available: http://www.R-project.org.

37. Holt RD, Polis GA (1997) A theoretical framework for intraguild predation. Am Nat 149: 745–764.

38. Holt RD, Huxel GR (2007) Alternative prey and the dynamics of intraguild predation: Theoretical perspectives. Ecology 88: 2706–2712.

39. Grason EW, Miner BG (2012) Behavioral plasticity in an invasional system: Non-native whelks recognize risk from native crabs. Oecologia 168: 105–115.