Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density?

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Abstract: Food mixing behavior of omnivorous consumers can be difficult to predict. We undertook an enclosure experiment to explore the effects of ontogenetic stage and density on food choices of the omnivorous signal crayfish, *Pacifastacus leniusculus*. Juvenile or adult crayfish were placed in in-stream enclosures for 6 weeks at three different densities. Gut and stable isotope analyses were used to determine the food sources utilized by these organisms. In addition, we analyzed the guts of both adult and juvenile crayfish from outside the enclosures to account for experimental effects. We found few differences in the gut contents between juvenile and adult crayfish from either inside or outside the enclosures, as the majority of food consumed by both ontogenetic stages and all densities was allochthonous detritus. Stable isotope results indicate that crayfish of both ontogenetic stages were relying primarily on detrital biofilms for nutrition, despite an additional laboratory experiment showing that growth would be far more rapid on invertebrates. The dietary choices made by *P. leniusculus* in its native environment seem to be primarily affected by factors other than the nutritional value of food sources, contrary to expectations that food mixing behavior of omnivores should be based on this factor alone.

Résumé : Le comportement de mélange alimentaire chez les consommateurs omnivores peut être difficile à prédire. Une expérience en enclos nous a permis d'explorer les effets du stade ontogénique et de la densité sur les choix alimentaires de l'écrevisse omnivore *Pacifastacus leniusculus.* Nous avons placé des écrevisses juvéniles ou adultes dans des enclos dans un cours d'eau pendant six semaines à différentes densités. Des analyses du tube digestif et des isotopes stables ont servi à déterminer les sources de nourriture utilisées par ces organismes. De plus, nous avons analysé les tubes digestifs d'adultes et de juvéniles provenant de l'extérieur des enclos afin de tenir compte des effets du protocole expérimental. Il y a peu de différences entre les contenus des tubes digestifs des jeunes écrevisses et des adultes, tant de l'extérieur que de l'intérieur des enclos, puisque la plus grande partie de la nourriture consommée par les deux stades ontologiques, à toutes les densités, est constituée de détritus allochtone. Les résultats de l'analyse des isotopes stables indique que les écrevisses des deux stades ontogéniques dépendent principalement des biofilms sur le détritus pour leur alimentation, bien que des expériences supplémentaires en laboratoire indiquent que la croissance serait beaucoup plus rapide s'ils se nourrissaient d'invertébrés. Les choix alimentaires faits par *P. leniusculus* dans son habitat indigène semblent affectés surtout par des facteurs autres que la valeur nutritionnelle des sources de nourriture, ce qui est contraire à nos attentes que le comportement de mélange alimentaire devrait se baser sur ce seul facteur.

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Introduction

Omnivory has been recognized as an important aspect of both aquatic and terrestrial food webs (Polis and Strong 1996; Pringle and Hamazaki 1998). In contrast with specialist consumers, omnivores may have large direct and indirect impacts on several trophic levels and consequently affect ecosystems in ways that are difficult to predict. Omnivores can have diets that include plants, animals, and detritus and therefore will simultaneously have negative influences on both numbers of prey and habitat availability for prey (Dorn and Wojdak 2004). To complicate things further, the diet of an omnivore may not remain consistent either through its life or within specific life history stages. Comprehension of the factors that drive food selection behavior of omnivores is central to understanding their potential roles in the ecosystem (Singer and Bernays 2003). Here, we focus on two factors that could potentially affect the food choices made by omnivores: the ontogenetic stage of individuals and the density of their populations.

Omnivorous organisms can undergo shifts with respect to food preference at certain times within their life cycles. Such

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shifts, prevalent in organisms such as fish, amphibians, and invertebrates, may render different life history stages of the same organism ecologically different contributors to the ecosystem (Polis 1984; Olson 1996; Hjelm et al. 2000). Life history omnivory (Pimm and Rice 1987) is prevalent in the aquatic environment (Tavares-Cromar and Williams 1996) and generates the potential for organisms to have disparate effects on their surroundings at different life history stages. Stream-dwelling crayfish are described as having an ontogenetic shift in their food preference from juveniles to adults. Juvenile crayfish are primarily carnivorous (e.g., *Astacus astacus*, Abrahamsson 1966; *Orconectes rusticus*, France 1996; *Pacifastacus leniusculus*, Guan and Wiles 1998), whereas adults are omnivorous (e.g., *O. rusticus*, Lodge et al. 1994; *Orconectes luteus* and *Orconectes punctimanus*, Whitledge and Rabeni 1997; *Cambarus bartonii*, Schofield et al. 2001). Evidence for this shift comes from work done on dietary (gut) analysis in crayfish of several genera (e.g., *Pacifastacus*, Guan and Wiles 1998; *Paranephrops*, Parkyn et al. 2001; *Procambarus*, Correia 2003) and provides the possibility that there may be a strong ontogenetic component to the effects of crayfish on the stream community.

In addition to the complicating factor of ontogeny, omnivores may alter their diet through density-dependent processes if resources are limiting or if there is strong intraspecific aggression. Svanback and Persson (2004) have shown that individual dietary specialization among populations of perch (*Perca fluviatilis*) fluctuates with the density of the population and is related to the degree of intraspecific conflict. Individuals, as well as the population, were prone to consume more generalized diets at higher densities, which concurs with research showing that increased levels of competition will result in suboptimal resources becoming more valuable (Bolnick 2001). At present, little is understood about the variation in resource use within a species driven by density-dependent processes (Svanback and Persson 2004).

Stream-dwelling crayfish are likely subject to density dependence with respect to dietary choices, as they are known to achieve high densities in certain microhabitat types. Both juvenile and adult signal crayfish (*P. leniusculus*) are found in areas of stream with low flow and high allochthonous detritus buildup and are both intra- and inter-specifically aggressive (Guan and Wiles 1998; Pockl and Pekny 2002; Nakata and Goshima 2003). One may postulate that diet will be altered in some individuals in these areas of high density owing to increased rates of aggression. Such behavior-based dietary changes, coupled with ontogenetic changes, lead to a complex array of potential crayfish impacts on stream ecosystems.

The purpose of this study was to assess the dietary choices of juvenile and adult *P. leniusculus* at three densities in experimental enclosures. We predicted a preponderance of carnivory in juveniles at low density followed by increased ingestion of lower quality food resources and lower levels of gut fullness at higher densities. For adults, we predicted that detritivory would be prevalent at low density and that there would be an increase in the incidence of cannibalism by the largest individuals at high densities. In addition, we predicted a decrease in gut fullness of subordinate (smaller) adults at high densities resulting from a restriction of movement or inhibition by the largest individuals. Subsequent to the dietary analysis experiment, we undertook growth experiments in the laboratory to determine the effects on growth of the food choices made in the field, and thus the potential fitness consequences of these choices.

Methods

Study organism

Pacifastacus leniusculus, the only crayfish native to British Columbia, was used in these experiments. The natural range of *P. leniusculus* extends from the southern part of British Columbia (Hamr 1998) to the northern part of California (Elser et al. 1994) and east to parts of Utah and Montana (Johnson 1986; Sheldon 1989); however, this organism was widely introduced into many parts of Europe and Asia in the mid- to late 1900s to compensate for the loss of native European and Asian species caused by the crayfish plague (Abrahamsson and Goldman 1970; Svardson 1995). Apart from a small number of studies done in the 1970s in British Columbia (e.g., Mason 1975), most of what is known about the ecology of this organism is as an introduced species, including descriptions of the ontogenetic niche shifts in diet (e.g., Guan and Wiles 1998).

Field diet study

This experiment took place in Spring Creek (see descriptions in Richardson 1992; Reece and Richardson 2000; Negishi and Richardson 2003), a second-order stream located in the University of British Columbia's Malcolm Knapp Research Forest. This forest is located in southwestern British Columbia (49°18′40″N, 122°32′40″W) in the Coastal Western Hemlock biogeoclimatic zone. The riparian vegetation surrounding Spring Creek consists primarily of red alder (*Alnus rubra*) with a smaller representation of vine maple (*Acer circinatum*), while the dominant forest cover is largely Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*).

The enclosure experiment was carried out within a large run reach of the stream (approximately 100 m), with a depth of approximately 30 cm and a cobble–gravel substrate. The experiment took place from early June to mid-July 2002, at which time the stream temperature was between 8 and 12 °C.

Enclosures (1 m × 1 m × 60 cm) were constructed with 1.25 cm diameter PVC pipe and plastic hardware cloth (mesh size 1 cm^2) and dug into the streambed to a depth of 30 cm. Once the enclosures were embedded within the streambed, they were refilled with substrate to a depth that matched the level on the outside. This allowed for a full representation of food items larger than the mesh size to be included within the enclosures. Small items would also have been added in this manner and would additionally have been able to drift into the enclosures throughout the experiment. To standardize the leaf litter content within each enclosure, four 5-g leaf packs (red alder) were added and allowed to condition for 10 days prior to the start of the experiment. Senescent leaves had been collected from the riparian area of Spring Creek in the previous fall and air-dried in the laboratory. After weighing, leaves were rewetted and bound with wire-based garden ties and affixed to the inside of the enclosures.

Crayfish treatment densities were in the same range as natural field densities: one, two, or three adults (average orbital carapace length (OCL) of 32.5 mm) per enclosure or four, eight, or 12 juveniles (average OCL 18.5 mm corresponding to 2-year-old sexually immature juveniles (Mason 1975)) per enclosure. All six treatments were replicated in four complete blocks. Once all crayfish were placed into the enclosures, they were covered with galvanized steel hardware cloth (mesh size 1 cm^2) to allow for maximum exposure to light while preventing the crayfish from escaping or predators from entering. The experiment ran for a total of 6 weeks, during which time the sides and tops of the enclosures were brushed biweekly to prevent debris buildup.

Data collection

After 6 weeks, all crayfish were collected and immediately euthanized in carbonated water. They were frozen upon return to the laboratory to prevent further digestion of food items. In addition, five adult crayfish and five juvenile crayfish were collected from Spring Creek (~100 m downstream of the enclosures) and immediately euthanized and frozen in the same manner. These specimens were collected for gut analysis to assess potential effects of the enclosures on crayfish diet. We also collected five young-of-the-year (YOY) crayfish (average OCL 11.6 mm) to assess how their gut contents compared with those of the 2-year-old juveniles used in the experiment.

Gut content analysis

The foreguts of all crayfish from each treatment (apart from eight juvenile and 12 juvenile treatments where five specimens from each enclosure (n = 20) were used) were dissected out and analyzed for fullness and content. The contents of the foregut were used for this analysis, as they are more easily identifiable than the contents of the hindgut, which have been subject to further mechanical and chemical digestion. The methods of Parkyn et al. (2001) were used. The foreguts were dissected out of the crayfish, and all contents were then flushed through a 500-μm sieve and preserved in ethanol until subsequent analysis. All particles greater than 500 μm (0.5 mm) in size were counted and identified, allowing for more accuracy in calculating percentages of each component within the diet. The diets of the additional crayfish caught from Spring Creek were analyzed in a similar manner.

To avoid pseudoreplication from within the enclosures, gut content proportion data were averaged per enclosure and then arcsine square root transformed and assessed for statistical differences between different densities of each ontogenetic stage (e.g., to test for differences between the four-, eight-, and 12-juvenile treatment or the one-, two-, and three-adult treatment) using the multivariate analysis of variance (MANOVA) procedure in SAS version 8e (SAS Institute Inc. 2004). Apart from one food type for juvenile crayfish, no statistically significant differences were found between densities for either juveniles or adults (see Results section). Data from all density treatments for each ontogenetic stage were therefore pooled, resulting in a total of n = 12 for juvenile guts (based on a total of 56 individuals dissected) and n = 14 adult guts (based on a total of 24 individuals dissected). We then performed a MANOVA to compare the diets of juvenile versus adult crayfish. A second MANOVA was performed to compare the diets of crayfish (both adult and juvenile) from the experiment with those of crayfish caught directly from Spring Creek (n = 5 for the juvenile crayfish and n = 5 for the adult crayfish caught directly from Spring creek). The proportions of food types within the guts were the response measures, although the lowest proportion was left out of the analysis to avoid sum = 1. Gut fullness measures were assessed for statistical differences between different densities of each ontogenetic stage of crayfish from within the enclosures using the GLM (general linear model) procedure in SAS version 8e (SAS Institute Inc. 2004).

Stable isotope analysis

The use of stable isotope analysis in corroboration with gut content analysis provides for a more robust approximation of the diet, as it takes into account the food types that are assimilated into crayfish tissue, thereby eliminating overestimation of some diet items that have a long latency period in the gut. As there were no significant differences in the gut contents between juveniles in different density treatments and adults in different density treatments (see Results section), five individuals of each age category were selected at random for analysis of stable isotopes of carbon and nitrogen in addition to the five YOY juveniles caught directly from Spring Creek. Frozen tissue from the tail muscle was used for this analysis. Conditioned leaf litter and woody debris were collected from Spring Creek, well rinsed, and immediately frozen. The biofilm layer from 15 leaves and 15 chunks of woody debris were removed using a soft-bristled toothbrush and distilled water. The biofilm solutions were centrifuged and freeze-dried prior to homogenization with a mortar and pestle. Ten leaves and 10 pieces of woody debris were dried at 60 °C for 72 h and homogenized in the same manner. Chironomids (n = 50) and heptageniid (n = 20) and leptophlebiid mayflies (n = 20) were collected from Spring Creek, and the guts of the mayflies were dissected out before drying all organisms in a 60 °C oven for 24 h. Samples were then ground prior to stable isotope analysis. For the biofilm samples, leaves, wood, and invertebrates other than crayfish, two samples of the homogenized ground tissues were run for stable isotope analysis (n = 2). As these homogenized samples were not independent, they reveal subsampling error rather than the true individual-to-individual variation shown by the crayfish data. All samples were analyzed using a Finnigan Deltapluss mass spectrometer for measuring isotope ratios of carbon and nitrogen according to standard methods.

Laboratory feeding experiment

A feeding experiment was set up based on the results of the gut content analysis described above. Thirty 20-L aquaria were filled with water from Spring Creek (sieved at 63 μm) and attached to a system of aeration. Adult and juvenile crayfish were caught from Spring Creek and brought directly to the laboratory for the experiment. Each aquarium contained one crayfish to preclude any effects of competition between crayfish.

Treatment (food) types were based on the predominant food types found in the gut content analysis plus inverte-

brates: conditioned leaf litter, conditioned woody debris, and a mixture of live and dead invertebrates (Chironomidae (blood worms)). Leaves of red alder had been collected from the vicinity of Spring Creek the previous autumn and air-dried. Bundles of these leaves were placed in the creek at least 10 days prior to being used in the experiment to allow for conditioning. Crayfish were given two leaves per day (or more as needed). Woody debris was collected from the field as well and broken into 1 cm × 1 cm pieces to allow for crayfish handling. Crayfish were given at least four pieces daily to ensure a surplus of food. All leaves and wood were thoroughly washed prior to being placed in aquaria to remove any invertebrates or other debris. Invertebrates were provided to the crayfish twice daily; however, the aquaria were closely monitored to ensure that there were always enough worms to eat while not allowing for a buildup that might have altered aquarium pH, bacteria, or oxygen levels.

For the juvenile crayfish, there were a total of six replicates for each food source, and for adults, there were four replicates for each food source. The experiment was carried out from early May until the end of July 2003 at a constant water temperature of 16 °C. Aquaria were cleaned biweekly to remove debris and feces, and half of the water was replaced with freshly filtered water from Spring Creek. At each biweekly cleaning session, each crayfish was weighed and its OCL measured to assess growth.

Data analysis

Total weight gain and total change in OCL for adults and juveniles for each food source were analyzed separately using the GLM procedure in SAS version 8e (SAS Institute Inc. 2004). The ratio of OCL change to weight gain was also assessed in the same manner. For significant ANOVA results, post hoc least-squared means were calculated to determine which treatments differed from each other.

Results

Field diet study

Gut content analysis

A total of seven categories were discernable from the foregut contents. These were deciduous leaves, wood, macrophytes, conifer needles, invertebrates, crayfish parts/molts, and other (unidentifiable organic matter). Juvenile crayfish exhibited a difference in the amount of macrophytes in the diet between different density treatments ($F_{[2,9]} = 8.13$, $p = 0.0096$); however, this difference was not a result of increased or decreased density, as the highest levels of macrophytic material were found in the eight-juvenile treatment. Levels of macrophytes in the diet of juveniles in the low-density (four) treatment were the lowest, and those in the diet of juveniles in the high-density (12) treatment were in the midrange. As this difference in diet did not reflect a trend of altered diet with increased density, and there were no other significant differences in dietary composition between any of the density treatments for juveniles or adults, all juvenile data were pooled together ($n = 12$) and all adult data were pooled together ($n = 12$) prior to the comparison between juvenile and adult diets. In addition, there were no differences in gut fullness for adults or juveniles in any of the different treatments. Juveniles had significantly more leaves and needles in their diets (MANOVA: $F_{[1,22]} = 12.31$, $p = 0.002$, and $F_{[1,22]} = 13.10$, $p = 0.001$, respectively) (Figs. 1*a* and 1*b*) than adults. Juvenile guts also had significantly more insects than adult guts (MANOVA: $F_{[1,22]} = 17.59$, $p = 0.0004$); however, for both adults and juveniles, insects were among the smallest components of the diet (0.6% and 1.6%, respectively). Adult guts contained more crayfish parts/molts than juvenile guts (MANOVA: $F_{[1,22]} = 18.98$, $p = 0.0003$); however, the incidence of cannibalism did not increase with an increase in adult crayfish density, with one incident occurring in the two-adult treatment (reduced to one individual) and one incident occurring in the three-adult treatment (reduced to one individual).

There were no significant differences in dietary composition between individuals (either juvenile or adult) from within the enclosures and those caught directly from Spring Creek (Figs. 1*c* and 1*d*), with the exception of the proportion of insects found in the guts (MANOVA: $F_{[1,31]} = 7.04$, $p = 0.01$). There were fewer insects found in the guts of the crayfish caught directly from Spring Creek.

Gut content analysis of the YOY juveniles revealed that these individuals had a diet similar to that of all other crayfish assessed in this study. Woody debris was by far the most represented item in the diet of the crayfish followed by leaves, macrophytes, and needles (Fig. 1*e*). Insects were the least represented item in the diet, comprising around 0.5% of the gut contents.

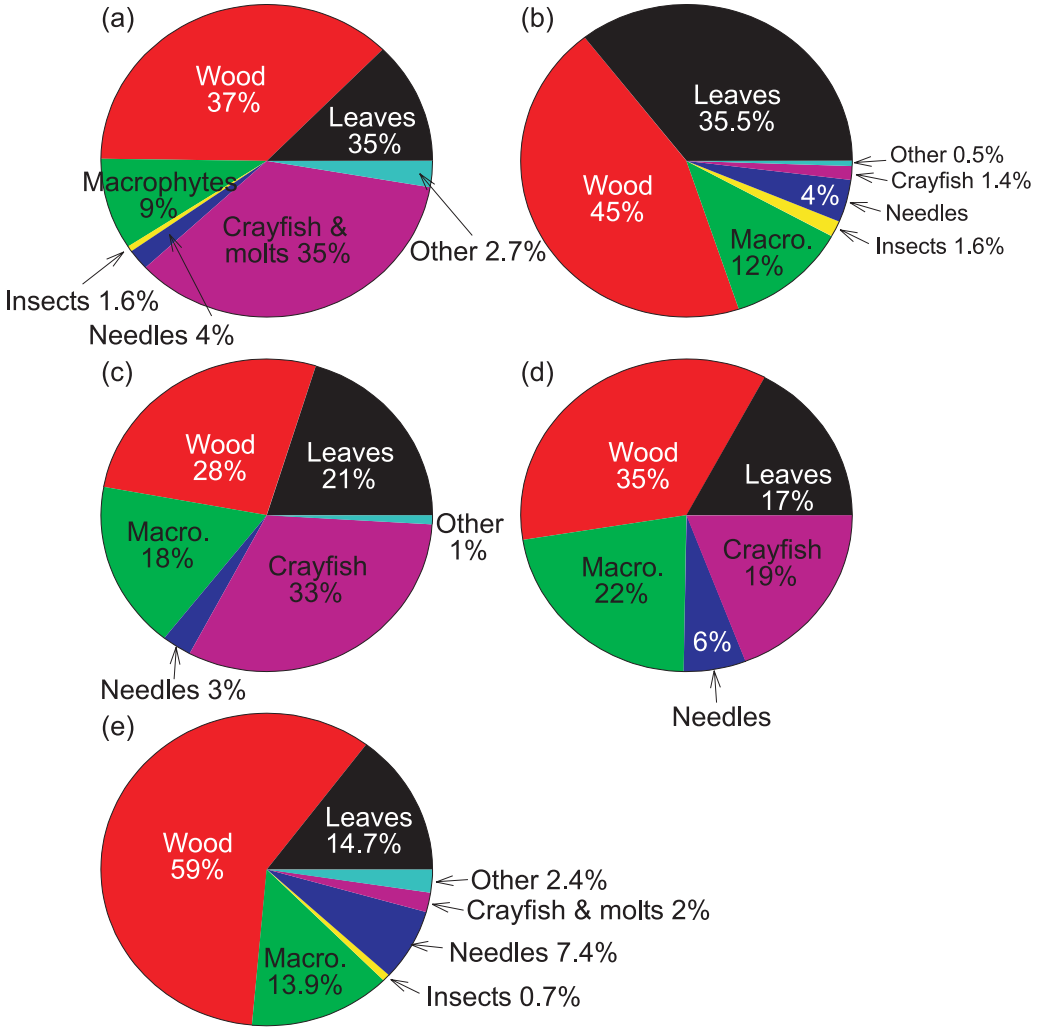
Stable isotope analysis

Stable isotopes of carbon and nitrogen did not vary to a great extent with crayfish ontogenetic stage (Fig. 2). Adult crayfish were slightly more depleted in nitrogen than juvenile or YOY crayfish; however, this difference was less than 1.0‰ and therefore not large enough to represent a complete change in trophic level (Parkyn et al. 2001). Adult and YOY crayfish were very similar in their carbon signatures, while juvenile crayfish were slightly more depleted. Crayfish were not found to be more nitrogen enriched than chironomids or heptageniid mayflies, indicating that they are on the same trophic level as these herbivorous grazers. Crayfish were more nitrogen enriched than leptophlebiid mayflies and all detrital samples. For both leaves and wood, the nitrogen and carbon signals varied between the biofilm scrapings and the entire fragments. Leaf biofilm was considerably more nitrogen enriched than the actual leaves (approximately 4‰), while wood biofilm was approximately 2‰ more enriched than wood. In both cases, this represents a change in trophic level (Peterson and Fry 1987).

Laboratory feeding experiment

Overall growth on the invertebrate diet was significantly higher for both adults ($F_{[2,9]} = 4.87$, $p = 0.03$) and juveniles ($F_{[2,15]} = 22.66$, $p < 0.0001$) compared with a diet of leaves or wood (Fig. 3). This same trend was evident for OCL increase. There was no significant difference between weight gain for either adults or juveniles being fed leaves or wood (least-squared means post hoc test $p = 0.3$ for adults and $p = 0.51$ for juveniles), while significant differences between invertebrates and detrital sources were observed for both adults ($p = 0.07$ for invertebrates versus leaves and $p = 0.01$

Fig. 1. Gut contents of adult, juvenile, and YOY signal crayfish (*Pacifastacus leniusculus*) from within the enclosures, as well as caught directly from Spring Creek. (*a*) Adults from within the enclosures; (*b*) juveniles (2YA) from within the enclosures; (*c*) adults caught directly from Spring Creek; (*d*) juveniles (2YA) caught directly from Spring Creek; (*e*) YOY caught directly from Spring Creek. Percentages shown are averages based on $n = 12$ for Fig. 1*a*, $n = 12$ for Fig. 1*b*, and $n = 5$ for Figs. 1*c*–1*e*.



for invertebrates versus wood) and juveniles ($p < 0.0001$ for invertebrates versus leaves and $p < 0.0001$ for invertebrates versus wood). In addition, there were no differences in the length gained per gram of weight gained (OCL change/weight change) for any of the treatments.

Discussion

Results from our gut content and stable isotope analyses do not support the ontogenetic niche shifts for crayfish diet described by other authors (e.g., Abrahamsson 1966; Lorman and Magnuson 1978; France 1996), despite the fact that a clear growth advantage for juvenile crayfish would be realized from incorporating animal prey into their diets. In fact, our results show that regardless of whether they were in enclosures or not, juvenile *P. leniusculus* consumed food types that were just the opposite of those purported to be of most nutritional value to them. In all cases, detrital matter was the largest component of the diet for both adults and juveniles. It was also the greatest component of the diet of the YOY crayfish that we assessed. The results of Guan and Wiles (1998) and Stenroth and Nystrom (2003) show *P. lenisuculus* to be a substantial predator on chironomids, simuliids, and ephemeropterans in lentic systems; however, our results did not reach the same conclusion. It may be important to note that the studies of both Guan and Wiles (1998) and Stenroth and Nystrom (2003) were done on *P. leniusculus* as an introduced species in Europe. Apart from one study (Mason 1975) on the feeding ecology of *P. leniusculus* in a small woodland stream in British Columbia, little is known about the ecology of these organisms in their native North American habitats. In addition, our results do not corroborate those of Mason (1975), who found a greater proportion of insects in the diets of both juvenile and adult crayfish.

Although juveniles have a physiological need for larger amounts of protein (Momot 1995; Paglianti and Gherardi 2004), it does not seem as though juvenile *P. leniusculus* in our system were getting substantial input of protein through animal sources in their diet. Whitledge and Rabeni (1997)

Fig. 2. Stable isotope signatures of carbon and nitrogen for three ontogenetic stages of signal crayfish (*Pacifastacus leniusculus*), as well as their possible dietary components. Points shown are means, and errors are standard deviations. Sample sizes range from $n = 5$ for each crayfish to $n = 2$ (based on ground samples of 50 chironomids, 20 mayflies, 10 leaves, and 10 pieces of woody debris). Biofilm samples ($n = 2$) are based on dried biofilm scrapings from 15 leaves or 15 pieces of woody debris. All samples were collected from Spring Creek.

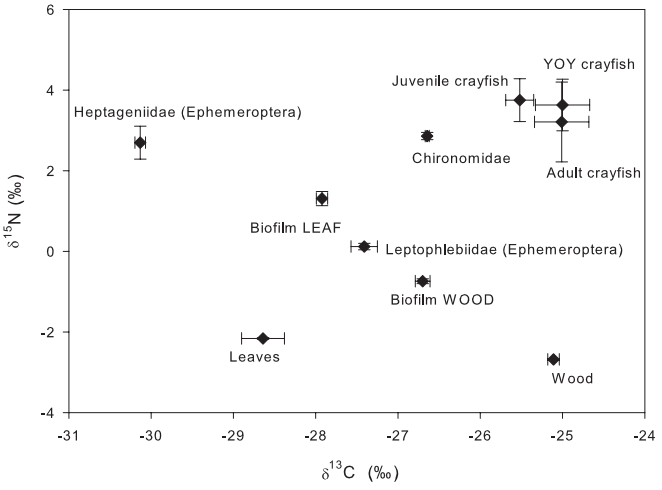
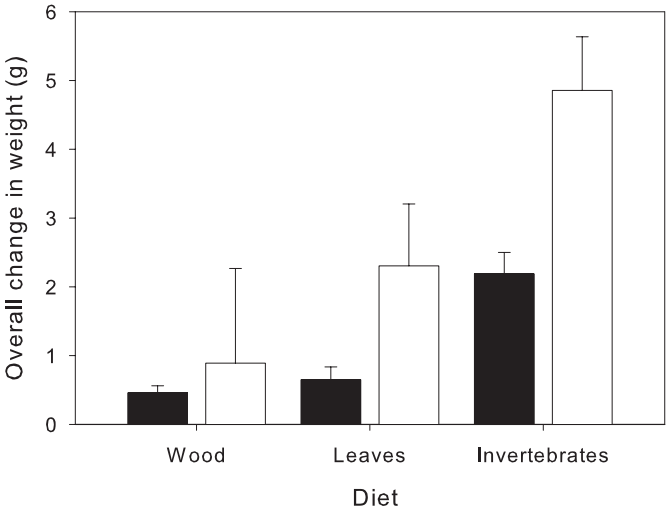


Fig. 3. Overall growth of both juvenile and adult signal crayfish (*Pacifastacus leniusculus*) raised on three different diets in a laboratory setting. Crayfish were housed in 20-L aquaria (one crayfish per aquarium). Food types (conditioned leaves, conditioned woody debris, or invertebrates (Chironomidae (blood worms)) were provided in excess for a period of 3 months. Mean overall weight change is shown for each food type for both adult crayfish (four replicates for each food type) and juvenile crayfish (six replicates for each food type). Error bars denote standard error.



determined the assimilation efficiencies of several food sources for the crayfish *O. luteus* and *O. punctimanus* and found that chironomids were much more efficiently assimilated (92%) than vascular plant detritus (14%). This indicates that a small portion of the diet may be composed of protein-based material to maintain an adequate growth rate. Despite the fact that the size and nature of insect matter may mean a shorter assimilation time in the crayfish guts, our sample size of 70 crayfish should have yielded some individuals who had recently processed insect matter if indeed they were doing so to a great extent in our system.

The assimilation efficiency results of Whitledge and Rabeni (1997) described above are corroborated by research showing that the stable isotope signature of crayfish reflects a diet of protein-based matter, while gut content studies show that adults ingest a substantial amount of vascular detritus (Parkyn et al. 2001; Hollows et al. 2002). However, our stable isotope results disagree with this conclusion as well. We would expect values of carbon and nitrogen to be substantially more enriched (as found by Parkyn et al. (2001) and Hollows et al. (2002)) if crayfish were predators in our system. We instead found that all ontogenetic stages were at the same trophic level as chironomids, despite the fact that chironomids have been hypothesized to provide the major protein-based food source for several crayfish genera including *Pacifastacus* (Guan and Wiles 1998; Hollows et al. 2002; Stenroth and Nystrom 2003).

Our results show crayfish to be approximately 2‰–3.5‰ more enriched than the biofilms on the wood and leaves, putting them one trophic level above these food sources (Peterson and Fry 1987) and indicating their role as detritivores. The carbon signatures of all crayfish most closely match those of wood and wood biofilm, agreeing with our gut content analyses and emphasizing the probable importance of these food types. However, interpretation of the dual stable isotope plot must be exercised with caution, as crayfish clearly do not assimilate insect and detrital food sources with the same efficiency (see discussion above), which violates a critical assumption of the use of stable isotopes to verify trophic position (Gannes et al. 1997). Although the results of the isotope plot must be interpreted with caution, our gut content analysis reaffirms the fact that all stages of *P. leniusculus* in its native habitat are utilizing detritus and detrital biofilms to a great extent in their diets. Whitledge and Rabeni (1997) suggested that the easily digested biofilms may contribute significantly to crayfish production given the large amount of detritus ingested by crayfish. Indeed, if more detritus was available to crayfish at a lower foraging cost, the energy intake may be higher on this resource despite the lower digestion efficiency. Several others suggested that the fungal/bacterial biofilm on allochthonous detritus is easily digestible and highly nutritious (e.g., McClain et al. 1992; Hollows et al. 2002). In addition, some researchers disagree that animal material is more efficiently assimilated by crayfish. Gherardi et al. (2004) showed that the assimilation efficiency of vascular detritus by *Austropotamobius pallipes* was 81%, agreeing with the results of Ilheu and Bernardo (1993) for *Procambarus clarkii* (73%). The former authors attributed this efficiency to the cellulolytic activity revealed in the hindgut of *A. pallipes*.

The results of our growth experiment showed no significant difference in the growth rate for either juveniles or adults fed leaves or wood. A steady ingestion of biofilm from either of these substrates could have provided a large portion of the energy required for growth, making the overall rates of weight gain for these two treatments indistin-

guishable from one another. While the detrital biofilm may indeed be a nutritious food source, it was clear that there were major fitness consequences (in terms of growth rate) for those crayfish raised on detritus versus those fed invertebrates. Both juvenile and adult *P. leniusculus* grew significantly more when fed on a diet of invertebrates, similar to the results of Paglianti and Gherardi (2004) for *A. pallipes* and *P. clarkii* raised on animal matter or detritus.

The question remains as to why we did not see any substantial ingestion of invertebrates by either adult or juvenile crayfish. Our only indication that *P. leniusculus* may attain some nutrition from animal sources in the field was the occurrence of cannibalism in the two- and three-adult treatments. Cannibalism by adult *P. leniusculus* was also documented by Guan and Wiles (1998). Large amounts of cover and habitat heterogeneity in the areas with higher crayfish density are clearly important features for adult crayfish survival. Despite the fact that crayfish and crayfish molt parts were found in the guts of several adult specimens, the stable isotope results do not reflect crayfish tissue as a major source of nutrition. This may indicate that the majority of food items in this category were molts (as opposed to actual crayfish tissue), ingested primarily for their calcium content. In addition, there was no indication that the occurrence of cannibalism was higher in the three-adult treatment compared with the two-adult treatment, as a single incidence of cannibalism was observed in both cases. It is possible that the occurrence of cannibalism in this species may be a by-product of several interacting factors, including habitat heterogeneity, resource availability, and timing of molts (which leaves newly molted crayfish in a highly vulnerable state).

Other than the single incidences of cannibalism in the two- and three-adult treatments, density of either juvenile or adult crayfish in the enclosures did not affect either gut fullness or gut contents. In addition, the smaller adults within the multiple adult treatments did not exhibit a difference in dietary composition or gut fullness from the larger adults in these treatments. This is not surprising given that all crayfish (except those preying on other crayfish) were exhibiting a large reliance on lower-quality food. Populations of *P. leniusculus* in Spring Creek are generally found in areas containing large amounts of conditioned allochthonous debris, so it is not surprising that this constitutes a major part of the diet. However, it is surprising that crayfish did not choose food sources (such as chironomids or other invertebrates) that would result in a higher growth rate.

Overall, this research has shown that the omnivorous signal crayfish *P. leniusculus* does not appear to undergo ontogenetic niche shifts in dietary preference that are commonly described for freshwater crayfish in its native North American habitat. Although there were some ontogenetic differences in the amounts of specific types of detritus ingested, all ontogenetic stages of crayfish appear to be primarily detritivorous, despite large protein requirements for fast-growing juveniles and a clear demonstration that an invertebrate-based diet would result in higher growth rate. In addition, insects were clearly able to colonize the enclosures (e.g., up to 700 individuals per enclosure have been counted; C.A. Bondar and J.S. Richardson, unpublished data) and were therefore available as food sources to the crayfish. Density of crayfish did not appear to change the nature of the choices selected by most crayfish (e.g., larger ingestion of lower-quality food at higher densities), indicating that presence of conspecifics was not responsible for the ingestion of vascular detritus over invertebrate food sources. These results show that neither ontogeny nor density has major effects on the ecological roles of crayfish in this stream community. Indeed, there were few differences in the insect communities within the enclosures that were dependent on either crayfish ontogenetic stage or crayfish density (C.A. Bondar and J.S. Richardson, unpublished data). Although these omnivores can potentially switch their diets to include food types from several trophic levels, there appear to be constraints preventing them from doing so to a great extent. Further work should focus on the mechanisms behind the selection of low-quality food in both juvenile and adult *P. leniusculus* in its native stream habitat.

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