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Non-native pelagic macro-invertebrate alters population dynamics of herbivorous zooplankton in a large deep lake.

Timothy J. Caldwell¹, Frank M. Wilhelm², Andy Dux³

¹Corresponding author: Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive MS 1136, Moscow, ID 83844. 717-575-7843.

Current affiliation: Department of Biology, College of Science, University of Nevada – Reno, 1664 N. Virginia St MS 314, Reno, NV 89557. 717-575-7843. Timothycaldwell@unr.edu

²Co-author: Frank M. Wilhelm, Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive MS 1136, Moscow, ID 83844. 208-885-7218. fwilhelm@uidaho.edu

³Co-author: Andy Dux, Principal Fisheries Research Biologist, Idaho Department of Fish and Game, 2885 W. Kathleen Ave. Coeur d’Alene, ID 83815, 208-770-3760. andy.dux@idfg.idaho.gov
Abstract

Establishment of non-native species can alter native species populations and re-structure lake food webs through complex trophic interactions such as intra-guild predation or classic trophic cascades. Although the freshwater shrimp, *Mysis diluviana*, is well known for its ability to impact both primary and secondary productivity through omnivorous feeding in aquatic ecosystems in which it has been introduced, empirical evidence of mysid consumption of zooplankton prey and associated impacts on cladoceran communities from the same lake is uncommon. Furthermore, recent literature has suggested that mysids may impact other zooplankton populations outside of cladocerans. To test the hypothesis that introduced mysids negatively affect the seasonal abundance of cladocerans in Lake Pend Oreille, a large and deep (348 km$^2$, max. depth - 352 m) lake in Idaho, USA, we quantified the seasonal diet and consumption rates of mysids, and compared them to cladoceran production rates. During isothermal conditions, mysids opportunistically fed on copepods, diatoms, and rotifers. However, during stratification, mysids strongly selected cladocerans. In addition, during isothermal conditions, mysids consumed 100% of cladoceran production and selected reproductive-sized individuals. We interpret this to show that mysids regulate the production of cladocerans when the lake is not stratified. The suppression of cladocerans in the spring coincides with the appearance of kokanee fry whose growth and survival may be negatively affected by the lack of abundant cladocerans, a preferred prey. This suggests a possible cascading effect to higher trophic levels.

Keywords: Mysis, Intraguild predation, Trophic cascades, Predator-prey interactions, Cladoceran


**Introduction**

In natural ecosystems, the size of populations in communities is regulated by a network of complex trophic interactions, one of which is predator-prey interactions (Hairston et al. 1960; Brooks and Dodson 1965; Polis and Strong 1996). This is especially well-recognized in lake ecosystems, in which predators influence food webs (Lane 1979; Carpenter et al. 2001). For example, a decline in zooplankton populations caused by a predator, can contribute to the decline of planktivorous fish or other species at higher trophic levels (Rieman and Falter 1981).

In systems with species that both compete with and prey upon another species, more complex interactions often occur resulting in a trophic triangle described as intra-guild predation (IGP, Polis and Holt 1992). For example, in Muriel Lake, British Columbia, sockeye salmon fry (*Onchorhynchus nerka*) compete with mysid shrimp (*Mysis* sp.) for cladoceran prey, but under the right conditions sockeye also consume mysids (Hyatt et al. 2005). Thus, trophic interactions are highly complex and unpredictable even in seemingly simple food webs (Hiltunen et al. 2012).

Lake ecosystems are often altered by large-scale extrinsic factors such as climatic change (Winder and Schindler 2004), nutrient loading (Carpenter et al. 1985) and anthropogenic activities, which undoubtedly influence the population regulation of aquatic organisms. However, it is the introduction or removal of predators that can cause immediate shifts in biological populations by initiating trophic cascades or creating a new IGP regime (Pace et al. 1999; Vander Zanden et al. 1999; Hyatt et al. 2005; Ellis et al. 2011). Therefore, it is critical that we examine and understand the complex and often unanticipated interactions that result from predator introductions, as their occurrence continues to increase in frequency and impact global biodiversity (Sala et al. 2000).
The freshwater shrimp, *Mysis diluviana* (previously *Mysis relicta*, Audzijonytė and Väinölä 2005), is an invertebrate omnivore with the ability to consume significant portions of zooplankton populations (Johannsson et al. 1994; 2001; Gal et al. 2006; O’Malley and Bunnell 2014). Described as opportunistic, mysids consume food from the benthos while they are on the lake bottom during the day (Van Duyn-Henderson and Lasenby 1986; Johannsson et al. 2001; Sierszen et al. 2011) and prey on pelagic zooplankton at night during diel vertical migration (DVM) (Johannsson et al. 1994; 2001; Viherluoto et al. 2000; Nordin et al. 2008). As young, or when zooplankton are scarce, mysids filter feed on algae (Bowers and Grossnickle 1978; Branstrator et al. 2000; Schindler et al. 2012) and some adults are cannibalistic on juveniles (Nordin et al. 2008). Although capable of consuming a variety of zooplankton species, the selection of prey by mysids is governed by prey availability and ease of capture, thus mysids prefer and typically select large, slow moving cladocerans (Cooper and Goldman 1980; Spencer et al. 1999; Nordin et al. 2008; Whall and Lasenby 2009), however recent research has suggested they can also impact copepod populations (Schindler et al. 2012). Because the abundance of cladocerans is seasonally dynamic in many lakes, omnivorous traits of mysids (Branstrator et al. 2000) are important to their energetics (France 2012; Schindler et al. 2012) and often result in additional impacts on food webs besides the reduction or elimination of zooplankton. These traits, coupled with predator avoidance (i.e., DVM; Beeton and Bowers 1982; Boscarino et al. 2007) make mysids a formidable predator in lentic ecosystems that play a significant role in structuring food webs.

Because of its high lipid content (Adare and Lasenby 1994), large size (1-2 cm), and its importance to fish throughout its native range (Wells and Beeton 1963; Issac 2010; Gamble et al. 2011a; 2011b), fisheries managers introduced *M. diluviana* into numerous lakes across the North
American Pacific Northwest and Scandinavia with the intent to increase fish production (Lasenby et al. 1986). Although, some introductions were successful (e.g., Sparrow et al. 1964), the majority resulted in the decline of some fish populations and caused significant changes to cladoceran population dynamics and entire lake food webs (Rieman and Falter 1981; Richards et al. 1991; Ellis et al. 2011).

Based on correlation between mysid abundance after their introduction and the seasonal decline of cladocerans, Rieman and Falter (1981) suggested that mysids in Lake Pend Oreille, Idaho, USA reduced the prey available to newly released kokanee fry which emerge from April to June. Only during stratification (July-Sep), when mysids are excluded from the epilimnion by warm water temperatures, did cladoceran abundance reach high densities after mysids became established in the lake (Rieman and Falter 1981). Chipps and Bennett (2000) used a general mysid bioenergetics model (Rudstam 1989), to estimate that mysids consumed 186 kg·ha$^{-1}$·year$^{-1}$ of cladoceran biomass in Lake Pend Oreille, which represents up to 70% of daily cladoceran standing stock. In contrast, cladoceran consumption by kokanee was estimated to be 45 kg·ha$^{-1}$·year$^{-1}$ (Chipps and Bennett 2000). However, neither of the conclusions reached by Rieman and Falter (1981) or Chipps and Bennett (2000) have been empirically tested using data from mysid gut contents. Given the variety of effects observed as a result of the introduction of mysids in other lake ecosystems (Schindler et al. 2012), empirical data from LPO are needed to support this hypothesis.

Here we use gut content analyses to examine the seasonal diet of mysids in Lake Pend Oreille, paired with simultaneous measurements of cladoceran production to empirically test the hypothesis that consumption of cladocerans by mysids limits cladoceran production.
Methods

Study Site:

Lake Pend Oreille (LPO), is a large (383 km$^2$) and deep (352 m maximum depth, 164 m mean depth) temperate oligotrophic (total phosphorus - TP < 7 µg·L$^{-1}$, chlorophyll $a$ < 5.5 mg·m$^{-3}$) natural lake, located in the panhandle of northern Idaho, USA (Falter and Ingman 2003). The main inflow to the lake is the Clark Fork River which enters LPO from Montana. The lake outlet forms the Pend Oreille River which flows west into the State of Washington (Fig. 1). The lake can be grossly separated into two major basins; the northern basin located to the north and west of the Clark Fork delta which is shallower (< 200 m maximum depth) than the southern basin located south of the Clark Fork delta (> 250 m maximum depth; Fig. 1). The water level of LPO is controlled at the outlet by the Albeni Falls Dam, for flood control, power production and augmentation of flow into the Columbia River Power System. Typically the lake level is maintained at maximum pool elevation from June to September, while in the winter it is dropped by up to 3 meters (Maiolie et al. 2006). The lake is typically thermally stratified between late June and September to an average depth of approximately 10-18m.

Each year from 1966 to 1970, 50,000 to 300,000 mysids from Waterton Lake, Alberta, Canada and Kootenay Lake, British Columbia, Canada were introduced to LPO (Rieman and Falter 1981). Annual surveys confirmed that mysids had established a self-sustaining population by 1972, reaching a density of 1,980 mysids·m$^{-2}$ by 1978 (Rieman and Falter 1981). During the 2000’s mysid densities fluctuated from approximately 300 to 1200 mysids·m$^{-2}$ in the 2000’s (Maiolie et al. 2006; Wahl et al. 2013); however, maximum densities of 2,471 +386 -334 individuals·m$^{-2}$ (geometric mean ± SE) were reported in 2009-2012 (Caldwell 2010; Caldwell and Wilhelm 2012). Following the introduction of mysids, kokanee salmon (Oncorhynchus nerka), declined precipitously after the 1960’s but have recovered in recent years to re-open a
recreational fishery (Wahl et al. 2013); however, the role of mysids in the decline is unclear.

Kokanee have multiple ecological roles in LPO and are utilized as a primary food source by bull trout (*Salvelinus confluentus*; Clarke et al. 2005), currently listed as threatened under the US Endangered Species Act of 1973, and by Gerrard-strain Rainbow Trout (*Oncorhynchus mykiss*). Native fish in LPO include Bull Trout, Westslope Cutthroat Trout (*Oncorhynchus clarki lewisi*), and Mountain Whitefish (*Prosopium williamsoni*). Kokanee salmon, Gerrard rainbow trout, lake trout (*Salvelinus namaycush*), Lake Whitefish (*Coregonus clupeaformis*), Smallmouth Bass (*Micropterus dolomieu*), Largemouth Bass (*Micropterus salmoides*), and Walleye (*Sander vitreus*) are all non-native fish that have become established in the ecosystem.

To characterize and examine the spatial and seasonal diet of mysids, we selected one site each in the north (north site; 161 m; N48°13.000’ W116°20.687’; Fig. 1), and south basin (south site; 287 m; N47°58.035’ W116°29.218’; Fig. 1). This selection was intentional to examine if mysid predation is similar in the two basins.

**Temperature profiles:**

To determine the seasonal thermal regime in LPO, we used an Amphibian logger connected to a Manta multi-probe sonde (Eureka Environmental, Austin, Texas, USA) which was lowered in 0.5 m increments from the surface to 35 m during each sampling event. Standard limnological isopleths were plotted to show the seasonal thermal regime in the lake at both sites. Additionally, we used a relative thermal resistance to mixing (RTRM) model (Kortmann and Henry 1981) to determine time periods and depths of stratification in LPO during 2009-2010. We also gathered historical profiles (1952, 1953) from Stross (1954), records (1976-1978, 1998-2003) from the Idaho Department of Fish and Game, and records (1989-1990) from the United States Geological Survey (USGS) to determine if the stratification patterns we observed were
similar to other years. The RTRM model was used to determine depth of stratification for all historical profiles during the months of August and plotted as a function of time.

Collection of mysid samples for gut content analysis and density:

To determine the density, and examine the diet of mysids in LPO, individuals were collected during the new moon phase at monthly intervals between May and November 2009 and at bi-monthly intervals between November 2009 and March 2010. To ensure that mysids had completed the upward part of the DVM cycle, all samples were collected around 12:00 AM, well after dusk. In addition, an echosounder was used to confirm that they had reached the upper 40 m of the water column. Triplicate samples of mysids were collected using a vertical net haul with a 1 m diameter hoop net with 1 mm-mesh and a 500 µm-screened cod end (Chipps and Bennett 1996). The net was lowered to 40 m, allowed to settle for several minutes, and then retrieved at approximately 0.33 m·s⁻¹ with the aid of an electric winch. Samples were preserved in 8% formalin until analysis. Samples were not collected at the north site during November due to inclement weather.

Mysid density at each site was determined by enumerating all mysids in each sample. Each mysid was measured from the tip of the rostrum to the base of the telson, aged, and sexed (Morgan 1980; Caldwell and Wilhelm 2012). Areal density (individuals·m⁻²) was calculated as the average number of the triplicate samples, divided by the area of the net (0.785 m²).

Volumetric density (individuals·m⁻³) was determined by dividing the average number of mysids collected in each of the three net hauls by the tow volume (31.6 m³).

Gut contents and prey composition indices:

Ten adult (> 10 mm) mysids from each month and site were haphazardly selected to evaluate gut contents. Mysids > 10 mm were selected because they are abundant throughout the
year, and because mysids < 10 mm are primarily herbivorous (Branstrator et al. 2000). With the aid of a dissecting microscope, mysid foreguts were dissected and spread onto a glass microscope slide with melted glycerol jelly, covered with a coverslip, and allowed to cool. Mysids from which guts were broken or spilled were not used for analysis and were replaced with new individuals. Gut contents of mysids were identified and enumerated with the aid of a compound microscope. Zooplankton were identified and counted based on the presence and number of mandibles (cf. Chess and Stanford 1998; Johannsson et al. 2001). To quantify the number of cladocerans eaten by mysids, mandibles were counted and divided by two using the assumption that two mandibles indicated that one cladoceran was consumed. This may slightly underestimate the actual number of cladocerans consumed.

The size of cladocerans eaten by mysids was estimated from a LPO-specific cladoceran body length to mandible length relationship. The body size of thirty cladocerans spanning the entire size range encountered in samples from LPO was first measured then followed by removal and measurement of the mandibles to the nearest 1 µm. Because mandibles of different cladoceran species could not be differentiated during the analysis of guts, the relationship between mandible length and total body length was established using all cladocerans (Daphnia and Diaphanosoma) encountered in LPO samples.

The number of prey eaten by mysids per night (individuals eaten·m⁻³·night⁻¹) was quantified on a monthly basis, using the equation:

\[ P_{lm} = i_m D_m G P_m \]

\[ \text{and } G P_m = G R T \times N L \]

\[ \text{and } G R T = 10.367 T^{-0.580} \]

\[ \text{and } N L = 24 - (\text{sunset time} - \text{sunrise time}) - 2 \]
where \( P_{im} \) is the number of prey \( i \) consumed per night (individuals eaten·m\(^{-3}\)·night\(^{-1}\)) in month \( m \); \( i_m \) is the mean number of cladocerans consumed in month \( m \); \( D_m \) is the density of large (> 10 mm) mysids (adult individuals·m\(^{-3}\)) in month \( m \); and \( GP_m \) is gut passages per night in month \( m \); \( GRT \) is gut residence time which is a temperature \( (T) \) dependent metabolic function; \( T \) is water temperature (°C) 1 m above the thermocline during month \( m \); 10.367 and -0.580 are regression coefficients; \( NL \) is night length in decimal hours; 24 is the number of decimal hours in a day; and sunset and sunrise times are in Pacific Standard Time converted to decimals. Two hours were subtracted to account for the time needed for ascent and descent (Chipps 1997). The equation for \( GRT \) was established with laboratory experiments using mysids from LPO (Chipps 1997, 1998). Water temperature just above the thermocline was used given the frequent observation of mysids at this depth via an echosounder (Caldwell, personal observation). Sunset and sunrise times were obtained from the United States Naval Observatory (USNO 2010).

Diet and feeding strategy was described using the prey-specific index \( (PSI) \), a modification of the Costello method (Amundsen et al. 1996). \( PSI \) is defined as the volumetric percentage a certain prey taxon comprises of all prey taxa for only those predators in which the prey occurs (Amundsen et al. 1996). \( PSI \) is calculated as:

\[
PSI_i = \frac{\sum S_i}{\sum S_{it}} \times 100
\]

(2)

where \( PSI_i \) is the prey-specific index of prey \( i \); \( S_i \) is the gut content volume comprised of prey \( i \) in individual mysids; and \( S_{it} \) is the gut content volume of only those predators with prey \( i \) present in the gut. \( PSI \) was plotted against the frequency of occurrence to determine prey importance and feeding strategy. Frequency of occurrence was calculated as:

\[
O = \frac{P_{it}}{P}
\]

(3)
where \( O \) is the frequency of occurrence, \( P_{it} \) is the number of predators with prey \( i \) present in their gut, and \( P \) is the total number of predators sampled. The volume of mysid foreguts is small (< 2 mm diameter) and direct measurement of the volume that a certain prey taxon occupied was difficult. Instead, we subjectively estimated volume using a “points” method (cf. Wilhelm et al. 1999). First, each gut regardless of fullness was assigned a value of ten points. Then all prey in each taxon found in the gut were visually assigned a value of points from 1 to 10, based on their contribution to the entire gut contents. For example, if diatoms and copepods contributed 70% and 30%, respectively, of an individual mysid gut, they received scores of 7 and 3. To describe the seasonal feeding strategy of mysids, \( PSI \) was calculated for each monthly sample.

Typically \( PSI \) is plotted against frequency of occurrence, showing prey importance, feeding strategy, and diet niche (see Amundsen et al. 1996 for a complete description). Instead, we plotted the product of \( PSI \) and frequency of occurrence as a function of time. This represented the seasonal diet of the mysid population and will be referred from here on as the “Population Prey Specific Index” (PPSI).

**Cladoceran density, size, and production:**

Triplicate vertical hauls of zooplankton were collected on each sampling occasion with a Wisconsin-style plankton net (30-cm diameter, 64 µm-mesh) during the same monthly intervals when mysids were sampled. The net was lowered to 20 m, allowed to settle for several minutes and retrieved at 0.33 m·s\(^{-1}\). Samples were preserved in 8% formalin until analysis. Each sample was brought up to 100 mL from which all zooplankton in a 5 mL sub-sample were enumerated and identified with the aid of a dissecting microscope. Cladocerans were identified to genus. Mean density (individuals·m\(^{-3}\)) of each taxon was estimated by back calculating from the subsamples and dividing the average of the three replicate samples by the tow volume.
To determine cladoceran size, 100 individuals (except in samples from the south site in October, and November which only contained 49 and 44 cladocerans, respectively) were measured by first haphazardly selecting a location in the petri dish containing the entire zooplankton sample and then measuring every individual encountered while working outward in an increasing spiral from the starting point. Each individual was measured from the top of the head to the inflection of the tail spine to the nearest 1 µm with the aid of a dissecting microscope fitted with a calibrated ocular micrometer. In addition the average body length of reproductive cladocerans was determined from gravid females encountered in samples (all gravid individuals were measured from each sample). The mean size of prey in mysid guts was compared to the mean size in the lake using a t-test for each month. All statistics were performed in SYSTAT® version 13.

Production of *Daphnia* and *Diaphanosoma* was calculated with the egg ratio method (Edmondson and Winberg 1971; Edmondson 1972; Paloheimo 1974). To estimate birth rate we used the equation described by Paloheimo (1974):

\[
b = \ln \left( \frac{C_t}{N_t} \right) + 1 \bigg/ D
\]

where \(b\) is the instantaneous birth rate, \(C_t\) is number of eggs, \(N_t\) is number of females, and \(D\) is the temperature-dependent egg development time in days. We used 2 days for \(D\) at 20 °C (Chipps 1997) and adjusted \(D\) along the normal Krogh curve (Edmondson and Winberg 1971) to match the observed water temperature. Instantaneous birth rate was multiplied by density for each species during each month, and production was estimated as births·m⁻³·d⁻¹. The sum of *Daphnia* and *Diaphanosoma* production was used to determine total cladoceran production (births·m⁻³·d⁻¹) for each month.
Comparison of mysid diet between sites:

To test if the diet of mysids differed between sites we used a nonparametric Kolmogorov-Smirnov (K-S) two-sample test, comparing the amount of each prey item consumed at each site.

K-S tests were run using SYSTAT® version 13.
Results

**Temperature profiles:**

Temperature profiles and the RTRM model indicated that thermal regimes and seasonal stratification of LPO was similar at the north and south sites (Fig. 2a, b). From October to April the lake was isothermal, with a temperature of approximately 4 °C. During late-April and May the surface water began to warm to ~8-10 °C. The lake was weakly stratified to a shallow depth of approximately 5 m during June but deeper (11-14 m), strong stratification did not occur until July and lasted until September, with temperatures reaching 23 °C at the surface, while the epilimnion was about 20 °C (Fig. 2a, b). Historical thermal stratification patterns (1954-2003) for August in LPO were similar to our observations in 2009 (Fig. 2c, d). The average thermocline depth between 1954 and 2010 at the north and south sites was 11.5 m (range 6-16 m) and 13.5 m (range 7-18 m), respectively (Fig. 2c, d).

**Gut contents and prey composition:**

Mysid diet varied seasonally (Fig. 3a, b), but was similar between sites (Table 1). A significant portion of the mysid gut content consisted of ‘other’ material that was amorphous, and unidentifiable (Fig. 3a, b). The highest volume of ‘other’ material occurred in October ranging from 100% to 84% at the north and south sites, respectively (Fig. 3a, b). During mid-to late spring (May and early June) mysid gut contents included diatoms, pollen grains, rotifers, and copepods which contributed up to 42% and 35% of gut contents at the north and south sites, respectively (Fig. 3a, b). Pollen was more prevalent in the diet of mysids at the south site in May and June (21% and 6%) compared to the north site (0% and 5%; Fig. 3). During July, rotifers were the most abundant prey in mysid gut contents, contributing approximately 31% and 60% to the gut volume at the north and south sites, respectively (Fig. 3a, b). Diatoms generally
contributed a higher percentage of material to gut contents at the north site than at the south site (Fig. 3a, b).

Cladocerans contributed < 1% to mysid gut contents during May, June and July (Fig. 3a, b) while the lake was isothermal, however, after stratification in August and September, mysids at both sites strongly selected cladocerans (Fig. 3a, b). During September, cladocerans contributed 70% and 82% to gut contents at the north and south sites, respectively (Fig. 3a, b). After the breakdown of stratification (October to March) mysids returned to a cosmopolitan diet, (Fig 3a, b). In summary, mysids were generalist feeders when the lake was isothermal and had a high within-phenotype component (Amundsen et al. 1996) and broad niche width, while during stratification, mysids strongly selected cladocerans.

**Cladoceran density and production:**

Cladocerans in lake samples were rarely observed when the lake was isothermal (Table 2). Interestingly, we noted the occurrence of cladoceran remains in the gut contents of mysids approximately 1 month before we obtained any in zooplankton tows. Total cladoceran density at the north site increased from 114 ± 57 (mean ±SE) individuals·m$^{-3}$ in July to 4941 ± 250 individuals·m$^{-3}$ in August, then decreased until November when they were no longer detected in lake samples (Table 2). A similar trend was observed at the south site (Table 2). The cladoceran community consisted of *Diaphanosoma* and *Daphnia* spp. *Diaphanosoma* was more abundant than *Daphnia* spp. during July and October, while *Daphnia* spp. made up > 85% of all cladocerans in August and September at both sites (Table 2). When the lake was isothermal (October- July), *Diaphanosoma* was detected at low densities (< 24 ± 22 individuals·m$^{-3}$) during January at the north site, and November at the south site.

Cladoceran production was low during late spring and early summer (May-July) but peaked at > 2000 births·m$^{-3}$·d$^{-1}$ in August at both sites (Table 2). In September, production
decreased to 505 and 843 births·m$^{-3}·d^{-1}$, at the north and south sites, respectively (Table 2). Production continued to decrease during October, and was < 120 births·m$^{-3}·d^{-1}$ at both sites (Table 2). *Diaphanosoma* made up > 90% of the total cladoceran production during July, October, and November at both sites (Table 2). In August and September *Daphnia* spp. accounted for > 80% of overall cladoceran production (Table 2). During January gravid cladocerans were not found in any samples, thus production was set to 0 births·m$^{-3}·d^{-1}$.

**The effects of mysid consumption on cladoceran production:**

Because we found cladocerans in the gut contents of mysids during some time periods when they were not detected in the lake, we calculated the production of cladocerans as zero. However, the consumption of cladocerans by mysids indicates that some production occurred, but without several variables required by the egg:ratio method (Edmondson and Winberg 1971; Edmondson 1972; Paleheimo 1974) we could not calculate a production rate. Because cladoceran parts were observed in mysid guts, we assumed that mysids consumed close to 100% of the cladoceran production during these time periods.

In May and June we calculated that mysids consumed between 9 and 11 cladocerans·m$^{-3}·night^{-1}$, respectively (Table 2), at the north site. No cladocerans were found in mysid guts in May at the south site, while we calculated that 5 cladocerans·m$^{-3}·night^{-1}$ were removed in June (Table 2). During stratification (July – September) mysids consumed considerably more (21-651 cladocerans·m$^{-3}·night^{-1}$) cladocerans at both sites (Table 2). Mysid consumption of cladocerans decreased at both sites between October and November, and during winter few cladocerans (0-5 cladocerans·m$^{-3}·night^{-1}$) were detected in mysid guts (Table 2).

Mysids removed 100% of cladoceran production at both sites in early spring (May-June north site; June-July south site; Fig.4a, b). The percentage of cladoceran production consumed
by mysids at both sites declined to 14-40% (depending on the month) once the thermocline
deepened (August or September). At the north site, mysids consumed 100% of cladoceran
production during September but only about 20% of production in October (Fig. 4a). At the
south site, consumption of cladoceran production by mysids returned to 100% in October upon
the return of isothermal conditions (Fig. 4b). March was the only month when cladocerans were
not detected in the lake or consumed by mysids, thus both consumption and production rates
were zero.

Our estimated percentage of cladoceran production consumed by mysids in LPO was
approximately 11 to 30% higher than previous estimates using a bioenergetics approach (Table
3). Our estimates were similar to those of Johannsson et al. (1994) for Lake Ontario where they
estimated that mysids consumed up to 110% of zooplankton production (Table 3). This is in
stark contrast to Gal et al. (2006) who estimated that mysids only consumed up to 4% of
zooplankton production in Lake Ontario. We were surprised to find only three studies that
quantified mysid consumption as a fraction of zooplankton production or standing stock (Table
3), however, numerous studies have quantified mysid clearance rates and examined prey
densities before and after mysid introductions (see discussion).

**Size of cladocerans selected by mysids:**

At the north site, the mean size of cladocerans increased from 1072.92 ± 25.81 (mean ±
SE) µm in July, to 1507.33 ± 42.32 µm in September, then decreased to 1269.74 ± 25.33 µm in
October (Fig. 5). Similarly, the mean size of cladocerans at the south site increased from 895.37
± 25.86 µm in July, to 1433.42 ± 34.06 µm in September, and then decreased to 1304.12 ± 33.92
µm in October (Fig. 5). The mean size of gravid cladocerans increased from 999 ± 34 µm to
1660 ± 47 µm and 1118 ± 28 µm to 1665 ± 46 µm at the north and south sites, respectively, from July to September (Fig. 5). No gravid individuals were observed in October.

Cladoceran body size was positively related ($R^2 = 0.80$, $n = 33$, $P = < 0.001$) to mandible length and described by the equation:

$$TBL = 9.69(ML) + 926.63$$

where $TBL$ is total body length of cladocerans in µm; $ML$ is mandible length in µm; and 9.69 and 926.63 are regression coefficients. This relationship was used to estimate the size of cladocerans consumed by mysids.

At the north and south sites, cladocerans in mysid gut contents were significantly larger than those in the lake during July, while they were similar in August, September and October (Fig. 5). Mysids also consumed cladocerans that were significantly larger than the average size of gravid females detected in the lake during July, but not in August or September (Fig. 5). After turnover, the size of gravid females declined to <1400 µm in October at both sites (Fig. 5).
Discussion

The impacts of mysid predation on the cladoceran population

Classic food web theory and hypotheses suggest that predators often regulate and control population dynamics of herbivores and/or primary producers (Hairston et al. 1960; Brooks and Dodson 1965), however, the complete domination of certain trophic levels or species populations is rarely observed. The impact of consumption by a macro-zooplankter such as mysids on the production of lower trophic levels has been examined in several ecosystems, albeit, many of these were modeled using a bioenergetics approach (e.g., Johannsson et al. 1994; Chipps and Bennett 2000; Gal et al. 2006). Consumption rates of prey by predators are rarely equal to or greater than production rates of the prey (Yan et al. 1991). We provide direct empirical evidence including predation rates based on gut content analyses and simultaneous calculations of zooplankton production which suggests that predation by mysids in LPO is commensurate with cladoceran production rates until LPO becomes thermally stratified and the epilimnion is sufficiently warm to provide a refuge for zooplankton from mysids. To our knowledge, the impact of mysid consumption on zooplankton populations presented here is the greatest that has been directly quantified via empirical evidence (Table 3), however, the extirpation of cladocerans in other lake ecosystems after the introduction and establishment of mysids suggests it may occur in other lakes (Richards et al. 1991; Ellis et al. 2011). Our results provide empirical evidence suggesting that mysids are responsible for the reduction or extirpation of cladoceran populations in several lakes which received mysid introductions (Lasenby et al. 1986).

The intra-guild predation (IGP) theory (Polis and Holt 1992) is often supported by pelagic food webs that have mysid shrimp because they and planktivorous fish tend to consume similar prey, and often the fish can and do consume the mysids (Hyatt et al. 2005). However,
our research suggests that planktivorous fish (kokanee salmon) have little to no effect on the
mysid population, suggesting a more classic trophic cascade model (e.g., Carpenter et al. 1985;
Pace et al. 1999) with regards to the upper trophic levels of LPO. It should be noted that we only
collected one year of mysid diet and cladoceran production data and more complex intra-guild
effects may occur based on the variability in fish abundance or lake primary productivity among
years (Hyatt et al. 2005). However, an analysis of the LPO community using stable isotopes
does not indicate a strong link between kokanee and mysids, but that kokanee rely on
cladocerans and chironomids (Clarke et al. 2005).

Our empirical data on the seasonal diet of mysids lends support to various hypotheses and
inferences made about the LPO food web in the past. For example, Rieman and Falter (1981)
used historical zooplankton data to infer that the suppression of cladocerans was caused by the
introduction of mysids. Similarly, bioenergetic model simulations by Chipps and Bennett (2000)
suggested that the removal of cladoceran production by mysids in early spring and fall was
significant. Because mysids do not typically tolerate water temperatures above 17 °C (Rudstam
1989; Boscarino et al. 2007), their vertical migration does not extend into the epilimnion (20°C),
affording cladocerans a warm-water predator refuge from mysids. Thus, in the absence of
mysids, they can reach high densities in the epilimnion. It is likely that mysids make
metalimnetic excursions (Murtaugh 1981) to consume cladocerans. These short migrations into
the metalimnion to briefly feed in prey rich waters do not appear to impact the population of
cladocerans. Breakdown of thermal stratification in the fall removes the thermal barrier allowing
mysids access to cladocerans again. Because our data show that predation by mysids removes
100% of cladoceran production during this time period, we conclude that predation by mysids
also contributes to the collapse of the cladoceran population in fall.
Mysids have been described as “sloppy” feeders because they consume the soft portion of *Daphnia* while often discarding the hard and difficult to digest parts (i.e., exoskeleton, mandibles, claws; Grossnickle 1982; Smokorowski et al. 1998). Because we used the detection of mandibles to quantify the number of cladocerans consumed by mysids, our consumption estimates are likely conservative and underestimate the impact of mysid predation on cladocerans. Indirect effects, such as the consumption of resting eggs by mysids, may further limit the production of cladocerans (Lehtiniemi et al. 2009).

Mysids have been shown to reduce the biomass and change the community structure of phytoplankton and diatoms through direct consumption in other systems (e.g., Lindén and Kuosa 2004; Lehtiniemi et al. 2009; Schindler et al. 2012). During isothermal conditions in LPO (Nov-Mar), 26-48% of the mysid diet consisted of diatoms. The role of mysid consumption in the primary productivity of lakes has not been satisfactorily explored, given that adults are omnivorous and juveniles are primarily herbivorous (Branstrator et al. 2000) it certainly deserves further investigation.

The selection of large zooplankton by mysids suggests another mechanism by which they delay the onset of an abundant cladoceran population in LPO. At the onset of stratification, the mean size of cladocerans consumed by mysids was larger than the mean size of gravid cladocerans in the lake. Thus mysids removed the largest individuals which would cause declines in cladoceran production rates because reproductive output is related to cladoceran body size (Lynch 1980). In the absence of large individuals that are highly fecund, the cladoceran intrinsic rate of increase would be reduced, and lead to slow population growth in spring and early summer.

*Seasonal diet and prey selection:*
Cooper and Goldman (1980) hypothesized that the selection of prey by mysids depended on; i) availability of prey; ii) prey escape ability; and iii) efficiency of prey capture. In LPO, the diet of mysids was highly plastic, similar to that reported in other lakes (Schindler et al. 2012; O’Malley and Bunnell 2014). For example, during winter, when diatoms were abundant, they occurred in mysid guts at both sites in LPO. This is similar to other systems in which mysids also feed on diatoms (Viherluoto and Viitasalo 2001; Lehtiniemi et al. 2009; Schindler et al. 2012). While the density of copepods is high in LPO (Caldwell and Wilhelm, unpublished data) they did not contribute significantly to the gut contents of mysids, however they select for copepods elsewhere (Schindler et al. 2012; O’Malley and Bunnell 2014). Ramcharan et al. (1985) and Nero and Sprules (1986) suggested that the rapid escape response of copepods reduces the ability of mysids to efficiently capture them. This may also be the case in LPO.

Caldwell and Wilhelm (2012) observed little to no growth in mysids during the winter months (Nov – Mar) in LPO. The consumption of less preferred food sources (i.e., diatoms and copepods) may cause the allocation of energy to prey capture rather than to growth and reproduction (Nordin et al. 2008). We hypothesize that low water temperatures associated with winter, coupled with the re-allocation of energy to the capture of prey items (i.e., diatoms and copepods) reduced the efficiency of predation and slowed growth.

Cladocerans are slow moving, high in nutrient content (Anderson and Hessen 1991), and abundant during certain times of the year. It is likely because of these characteristics that mysids in LPO strongly selected cladocerans when available. Similar patterns have been observed in other lakes (Johannsson et al. 2001; Whall and Lasenby 2009). The correlation between the consumption of cladocerans presented here and increased rate of growth by mysids (Caldwell and Wilhelm 2012) suggests that cladocerans may be important prey to mysids.
Potential cascading effects of the mysid-altered zooplankton assemblage on fisheries:

The temporal delay of cladoceran abundance caused by mysid predation on cladocerans has been implicated in the reduction of kokanee salmon (*Onchorhynchus nerka*) stocks (Nesler and Bergersen 1991), and was hypothesized in LPO (Rieman and Falter 1981; Bowles et al. 1991). While all life-stages of kokanee may be influenced by the suppression of cladocerans by mysids, fry may be the most susceptible because their emergence in LPO occurs between April and June at which time they depend on the presence of cladocerans (Rieman and Bowler 1980). This coincides with the apparent complete suppression of cladoceran abundance by mysids (see above). Thus, kokanee fry likely emerge into a food-poor environment, in which their preferred prey is absent and they are forced to find alternate prey, survive on reserves until the lake stratifies and cladoceran abundance increases (although warm epilimnetic water temperatures may preclude kokanee from the epilimnion), or perish. An abundant food supply is known to be important to recruitment of kokanee (Paragamian and Bowles 1995; Clarke and Bennett 2004) and predation pressure by mysids on zooplankton may represent a bottleneck to the kokanee population.

Variables which control the diel vertical migration of kokanee and sockeye salmon fry (the anadromous form of *Onchorhynchus nerka*) are not well understood (Beauchamp et al. 1997; Stockwell and Johnson 1999; Scheuerell and Schindler 2012). If kokanee spend a disproportionate time below the thermocline, their growth and survival may be reduced by the lower abundance of zooplankton due to the presence of mysids (see above). Thus, studies by Clarke and Bennett (2002a; 2002b; 2004) which concluded that the presence of mysids did not negatively influence kokanee growth and survival in LPO or the zooplankton assemblage
available to them may have been confounded by the shallow (2 m) depth at which they were conducted. Spatial overlap between mysids, kokanee fry, and other life stages of kokanee, along with the effects of predation on cladoceran density, may be more significant below the thermocline and underestimated in this and other studies (e.g., Chipps and Bennett 2000; Clarke and Bennett 2002a; 2002b; 2004).

The introduction of mysids to LPO has had a multi-pronged effect on the population of cladocerans. We observed a classic trophic cascade mechanism in the upper trophic levels (kokanee and mysids) but also observed evidence for intra-guild predation by mysids on cladocerans and their primary forage base (diatoms). Based on empirical quantification of the gut contents of mysids, we concluded that; i) direct consumption by mysids was commensurate to production by cladocerans during isothermal conditions (spring and fall); ii) mysids exhibited diet plasticity associated with the seasonal distribution and abundance of prey; and iii) mysids selected large-sized gravid cladocerans. Because of the effects-mysids have on cladoceran density, kokanee growth and survival in LPO is likely negatively influenced by mysids. Specifically, the temporal overlap of the recruitment of kokanee fry and the suppression of cladoceran production by mysids may represent a food bottleneck to the kokanee population. These results suggest that certain non-native predators have the ability to significantly alter the production and temporal dynamics of lower trophic levels, causing lake-wide changes to food webs.

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Game provided occasional use of research vessels, especially in winter. Funding for this project was provided by the Bonneville Power Administration through a grant to the Idaho Department of Fish and Game. T.J. Caldwell was supported by scholarships from the International Association of Great Lakes Research, the Washington State Lakes Protection Association and the Idaho Chapter of the American Fisheries Society. We thank Dr. Marion Wittmann, Dr. Clinton Davis, Dr. Sudeep Chandra, Jason Barnes, and Annie Caires for constructive comments that improved the manuscript. We also appreciate constructive comments from the associate editor and one anonymous reviewer that greatly improved the manuscript.


Table 1: Results of nonparametric Kolmogorov-Smirnov tests comparing mysid diet between the north and south site in Lake Pend Oreille, ID.

<table>
<thead>
<tr>
<th>Test</th>
<th>Max difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocerans</td>
<td>0.111</td>
<td>1.00</td>
</tr>
<tr>
<td>Copepods</td>
<td>0.444</td>
<td>0.31</td>
</tr>
<tr>
<td>Diatoms</td>
<td>0.111</td>
<td>1.00</td>
</tr>
<tr>
<td>Pollen</td>
<td>0.333</td>
<td>0.67</td>
</tr>
<tr>
<td>Rotifers</td>
<td>0.556</td>
<td>0.11</td>
</tr>
<tr>
<td>Other</td>
<td>0.333</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Table 2. Cladoceran density (mean individuals·m⁻³±SE), production (births·m⁻³·d⁻¹), and the consumption (individuals consumed·m⁻³·d⁻¹) of cladocerans by mysids, with percent contribution from each species in Lake Pend Oreille, Idaho, from May 2009 to March 2010.

<table>
<thead>
<tr>
<th></th>
<th>North</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>Jun</td>
</tr>
<tr>
<td>Cladoceran Density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diaphanosoma (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total cladocerans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>Jun</td>
</tr>
<tr>
<td>Cladoceran production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diaphanosoma (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total cladocerans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mysid consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia (%)</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>Diaphanosoma (%)</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>Total consumption</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

https://mc06.manuscriptcentral.com/cjfas-pubs
Table 3: The consumption rate of cladocerans by *Mysis diluviana* in Lake Pend Oreille, Idaho, and other lakes in North America.

Consumption rate is presented as a fraction of production or standing stock of cladocerans. Depending on the study, consumption rates were calculated using either bioenergetics (B) or empirical diets (ED), while production (P) was estimated using the egg ratio method Paleheimo (1974). In cases where production was not calculated, standing stock was presented (SS). We retained the original units of consumption and production published by the authors of each study due to the variability of methods, depths sampled and conversion factors (i.e., dry weight to wet weight, etc.).

<table>
<thead>
<tr>
<th>Lake</th>
<th>Consumption</th>
<th>Production/Standing Stock</th>
<th>Units</th>
<th>Percent Consumed</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario*</td>
<td>0.5 (B)</td>
<td>0.031 - 1.327 (P)</td>
<td>kcal·m⁻²·d⁻¹</td>
<td>38-110%</td>
<td>Johannsson et al. 1994</td>
</tr>
<tr>
<td>Lake Pend Oreille (1994)</td>
<td>0.08 - 2.4 (B)</td>
<td>0.89 – 14.12 (SS)</td>
<td>kg cladocerans·ha⁻¹·d⁻¹</td>
<td>9-17%</td>
<td>Chipps and Bennett 2000</td>
</tr>
<tr>
<td>Lake Pend Oreille (1995)</td>
<td>0.06 – 2.8 (B)</td>
<td>1.2 – 4.0 (SS)</td>
<td>kg cladocerans·ha⁻¹·d⁻¹</td>
<td>5-70%</td>
<td>Chipps and Bennett 2000</td>
</tr>
<tr>
<td>Lake Ontario**</td>
<td>0.0026 – 1.3 (B)</td>
<td>0.51 - 3.08 (SS)</td>
<td>g·wet weight zooplankton·m⁻²·d⁻¹</td>
<td>1-4%</td>
<td>Gal et al. 2006</td>
</tr>
<tr>
<td>Lake Pend Oreille (South)</td>
<td>9 – 651 (ED)</td>
<td>0 - 2163 (P)</td>
<td>ind·births·m⁻³·day⁻¹</td>
<td>18-100%</td>
<td>This Study</td>
</tr>
<tr>
<td>Lake Pend Oreille (North)</td>
<td>5 – 346 (ED)</td>
<td>0 – 2262 (P)</td>
<td>ind·births·m⁻³·day⁻¹</td>
<td>32-100%</td>
<td>This Study</td>
</tr>
</tbody>
</table>

* These consumption/production rates and percentages are based on the production of the entire zooplankton community, and approximated from Figure 5 and Table 6 in Johannsson et al. (1994). We used values from the >200 m deep site to be comparable to the site depths in our study (>300m).

** These consumption rates, standing stock estimates and percentages are based on the production of the entire zooplankton community. Consumption, standing stock, and percentage consumed were taken from Table 2, Table 4 and Table 6, respectively in...
Gal et al. (2006). Production rates were estimated by adding values from depth categories for the deepest site during each season, we presented the range in (g·wet weight zooplankton·L⁻¹).
Figure 1. Map of Lake Pend Oreille, ID and surrounding areas, and north (N) and south (S) sampling locations (modified from Caldwell and Wilhelm 2012). Contour lines are at 50 m intervals.

Figure 2. Water temperature isopleths for Lake Pend Oreille, ID at the north (a) and south (b) sites during 2009-2010; and historical (1954-2005, and 2009) thermal stratification depths during August from the north (c) and south (d).

Figure 3. Seasonal population prey-specific index (PPSI) plot of *Mysis diluviana* as a function of time at the north (a) and south (b) sites in Lake Pend Oreille, Idaho, USA. Prey-specific index (Amundsen et al. 1996) was plotted as a function of time to describe the seasonal diet of mysids.

Figure 4. The percentage of cladoceran production (births·m⁻³·day⁻¹) removed by mysid consumption (individuals·m⁻³·night⁻¹) and the depth of stratification at the north (a) and south (b) sites of Lake Pend Oreille, Idaho. The depth of stratification for each month is plotted as dark horizontal bars.

Figure 5. Average size of cladocerans eaten by mysids (gut – black filled circles), gravid females present in the lake (lake – open squares), and gravid females in guts of mysids (gravid females – grey filled triangles) as a function of time in Lake Pend Oreille, Idaho, at the north (a) and south (b) sites. Horizontal bars beneath monthly data sets indicate statistical differences between size of cladocerans eaten and those present in the lake.
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279x361mm (300 x 300 DPI)
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