TROPHIC ECOLOGY OF INTRODUCED POPULATIONS OF ALASKA BLACKFISH (*DALLIA PECTORALIS*) IN THE COOK INLET BASIN, ALASKA

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TROPHIC ECOLOGY OF INTRODUCED POPULATIONS
OF ALASKA BLACKFISH (*DALLIA PECTORALIS*)
IN THE COOK INLET BASIN, ALASKA

A

THESIS

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By

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Abstract

Invasive fishes frequently change natural aquatic habitats due to predation and competition. The Alaska blackfish (*Dallia pectoralis*) is indigenous to some regions of Alaska but was illegally introduced to the Cook Inlet Basin in the 1950s. By the 1970s, fisheries managers expressed concern over possible ecosystem-altering effects of the blackfish introduction, especially in waterbodies containing popular sport fish. Descriptive food habit studies may assist fisheries managers in making decisions regarding management of non-native populations of Alaska blackfish. This project characterizes diet of three Cook Inlet Basin Alaska blackfish populations through stomach contents analysis. Shifts in diet across season, sex, and size of individuals from a lake, wetland pond, and stream are discussed using the Index of Relative Importance. Cook Inlet Basin Alaska blackfish consume similar invertebrate prey as native juvenile salmonids and stickleback, with major prey consisting of epiphytic/benthic dipteran larvae, gastropods, and ostracods. Piscivory, including cannibalism, is infrequent in these populations. Due to the high degree of dietary overlap with native fishes and stocked sport fish, and evidence that many Cook Inlet Basin waterbodies contain established populations of Alaska blackfish, fisheries managers should take actions to restrict the spread of blackfish through public awareness education, law enforcement, and funding for additional research.

An Alaska blackfish husbandry manual outlines closed-system rearing and artificial fertilization protocols useful to researchers and educators for keeping live Alaska blackfish in the laboratory and classroom, in order to add to our body of knowledge about this species.

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Acknowledgments

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Dedication

For Greg, Laura, and Emily

Glory be to God for dappled things–
For skies of couple-colour as a brinded cow;
For rose-moles all in stipple upon trout that swim;
Fresh-firecoal chestnut-falls; finches wings;
Landscape plotted and pieced–fold, fallow, and plough;
And all trades, their gear and tackle and trim.

Gerard Manley Hopkins

Introduction

Invasive species are second only to habitat loss as a cause of extinction of native species in the United States (Lassuy 1995, Wilcove *et al*. 1998). Biological invasions alter natural ecosystems (Drake *et al*. 1989) and were described as early as 1958 by Elton as a "significant component of human-caused environmental change." Due to the ecological and economic risks of biological invasions, U.S. Executive Order #13112 of February 1999 calls for multiple federal agencies "to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause" (Fed. Regist. 1999).

The establishment of non-native fish populations is of particular concern, as fish introductions are often correlated with reduction or extinction of native fishes due to predation or competition for resources (Brown 1989). In the United States, 71 native fish species are listed as threatened, endangered, or negatively impacted by introduced fishes (Wilcove and Bean 1994, Fuller *et al*. 1999), and resulting human economic losses are conservatively estimated at more than one billion dollars annually (Pimental 2007).

While most fish introductions occur in warmer climates, Alaska lists 14 introduced fishes within its boundaries (McClory and Gotthardt 2008), including northern pike (*Esox lucius*; family Esocidae) and Alaska blackfish (*Dallia pectoralis*; family Esocidae; hereafter blackfish)–two species that are native to some regions of the state but introduced to others by people. In southcentral Alaska, introduced pike have destroyed popular sport fisheries by preying on stocked trout and salmon (ADF&G 2008), appeared to have altered the abundance and distribution of native fishes (Haught and von Hippel 2011), and likely caused the extinction of at least one rare phenotype of threespine stickleback (*Gasterosteus aculeatus*) (Patankar *et al*. 2006, von Hippel 2008).

Introduced blackfish now inhabit numerous lakes, ponds, and streams in the Cook Inlet Basin of southcentral Alaska (Morrow 1980, Stratton and Cyr 1997, Mecklenburg and Mecklenburg 2002, personal observation), and fisheries managers express concern over possible competition and predation by blackfish impacting native and stocked salmonids (Trent and Kubik 1974, Hepler and Bowden 1986). A previous study in their introduced range indicated substantial salmonid prey in blackfish gut contents (Chlupach 1975). In 1972, Jewel Lake (Anchorage) was rotenoned to eradicate a large blackfish population; however, blackfish differentially survive rotenone treatments, although the mechanisms are not understood. Some young-of-the-year blackfish from Meadow Lake (Anchorage) perished in rotenone concentrations greater than 0.004 ppm (see list of abbreviations, Appendix A), while others recovered (Chlupach 1975). Cheney Lake (Anchorage) was rotenoned during winter, 2011, to eradicate invasive northern pike; the following spring thousands of live and dead blackfish were captured in gillnets (K. Dunker, ADF&G, personal communication).

Fish diet analysis is an effective tool for understanding the impacts of introduced fishes on aquatic ecosystems (Garvey *et al.* 1998, Vander Zanden *et al.* 2000, Chipps and Garvey 2007). A particular fish's food habits reveal its trophic position within the overall food web (Pauly *et al.* 1998, Stergiou and Karpouzi 2002). Diet overlap can indicate potential resource competition between introduced and native species. The number of prey types in a fish's diet helps to define specialist versus generalist feeders, while spatial and temporal diet shifts highlight opportunistic, flexible feeding strategies. Trophic interactions including feeding habits can help reveal the extent of ecosystem alteration by introduced fishes, thereby providing useful information for ecosystem-based management (Pauly *et al.* 1998, Bachok *et al.* 2004, Stobberup *et al.* 2009).

Species Overview

Despite its establishment over the past 60 years, little is known about the ecology of blackfish within the Cook Inlet Basin. A brief synthesis of blackfish biology, life history, and physiology data gleaned from fewer than 20 articles published on the species is provided here as an introduction to this study.

The blackfish is a small fish endemic to fresh waters of Beringia. Its natural range extends from 55° to 72° N latitude on the Chukchi Peninsula of eastern Siberia, across western Alaska from the Colville River to the Alaska Peninsula, and inland through the Yukon-Tanana drainage to Fairbanks (Mecklenburg and Mecklenburg 2002). Introduced blackfish populations are found on Saint Paul Island in the Bering Sea and in southcentral Alaska including the Kenai Peninsula and Matanuska-Susitna Valley within the Cook Inlet Basin. Interestingly, there are no blackfish in Canada, although in 1956 they were introduced into Ontario farm ponds in hopes of starting a recreational fishery; all blackfish perished the first winter (Scott and Crossman 1973).

The blackfish is cryptically colored with a dark greenish-brown patterned back and sides and a light-colored belly. A short snout bears a protruding lower jaw and large mouth with small teeth on the mandible, premaxillae, vomer, and palatines (Mecklenburg and Mecklenburg 2002). Fins are light or white-edged, and the edges may turn red as an apparent stress response when fish are handled (personal observation). Wide pectorals, for which the fish derives its scientific name, enable slow sculling and maneuvering through waterweeds. Blackfish are normally "sluggish" bottom dwellers (Scholander *et al.* 1953) but can ambush prey in rapid bursts at temperatures as low as 3–5° C (Hanzely 1957).

Blackfish have small, stubby, toothed gill rakers–typically characteristic of benthic feeders, although this correlation is disputed by some as the "gill raker myth" (Gerking 1994). Pyloric caeca are absent. The physostomous swim bladder is well developed and enables the fish to orient at a 45° angle in the water column in search of predators and prey overhead (personal observation; see Fig. 1). A short Z-shaped gut (Fig. 2) consists of an esophagus, a straight stomach–also found in northern pike, but considered rare for predatory fish in general which usually have a U-shaped or Y-shaped stomach when present (Wilson and Castro 2011)–and short intestines.

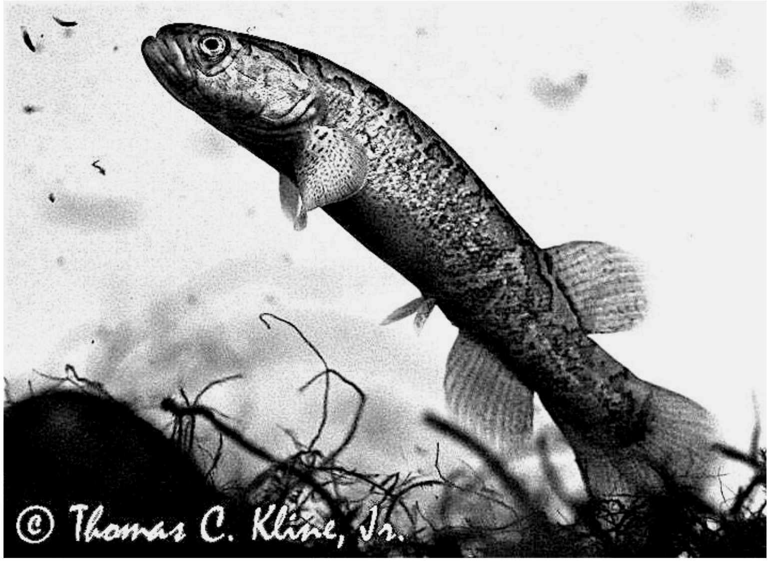

Figure 1. Adult blackfish in laboratory aquarium. Fish is feeding on previously frozen *Chironomid* larvae. Photographed in the von Hippel Lab, UAA, by Dr. Thomas C. Kline, Jr., copyrighted and used with permission.

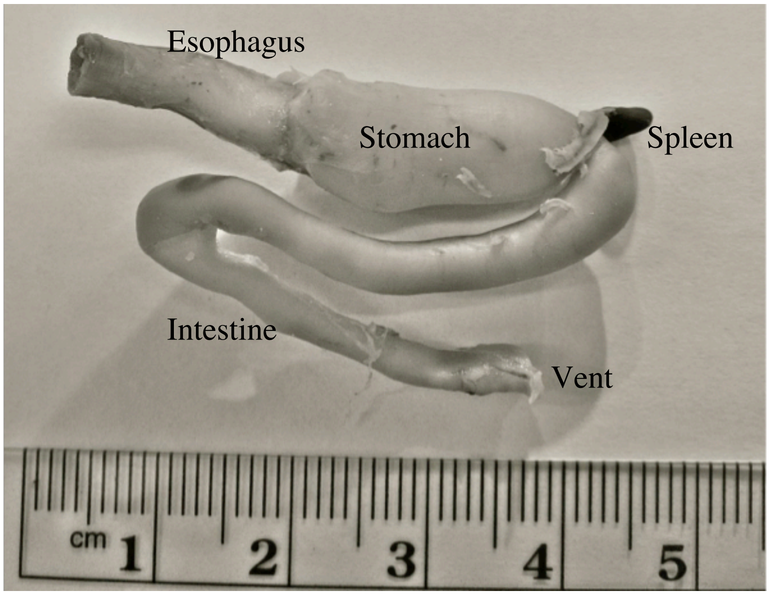

Figure 2. Blackfish gut. Dissection is from a preserved adult specimen.

Life history traits have been documented for two western/interior Alaska populations. Lake Alegnagik blackfish reach sexual maturity at age 2–3 (Aspinwall 1965). River residents are potadromous–migrating within freshwater habitats, moving out of wintering grounds in springtime when temperatures increase by 10–15° C. Adults paddle through dense wetland grasses to spawn upstream in small side channels or shallow lakes during summer (Blackett 1962). Lake Alegnagik blackfish are short-season spawners during a two-week period in July (Aspinwall 1965), while Big Eldorado Creek (Tanana River drainage) blackfish appear to spawn throughout the summer (Blackett 1962). Ovaries contain two egg types: 2.0 mm yellow-colored eggs for the present spawning season and 1.0 mm colorless eggs assumed to be for the following season's spawning (Aspinwall 1965). Fecundities range from 100–300 eggs, depending on fish size (McPhail and Lindsey 1970). Spawning behavior and location has not been documented. Hatch occurs within 9 days at 12.2° C, and larval growth is rapid during the first summer, to 20 mm by September (Aspinwall 1965). Total length averages 20 cm, although specimens from Anchorage have reached 33 cm (Morrow 1980).

Blackfish are legendary for their adaptations to life in Arctic and Subartic waters. They can tolerate living in high densities in small tundra pools (Morrow 1980). They exhibit extreme cold tolerance, although numerous anecdotes of fish freezing and then being thawed out alive are unsupported by laboratory investigations (Scholander *et al.* 1953, Scholander *et al.* 1957). A vascularized esophagus functions as an air-breathing organ and is known in only two other teleosts, the shanny (*Lipophrys pholis*) of northern Europe (Laming *et al.* 1982) and the Asian swamp eel (*Monopterus albus*), which is invasive in the southeastern United States (Liem 1967, Liem 1987, Fuller *et al.* 1999). Facultative air breathing enables blackfish to survive under hypoxic conditions, such as in warm shallow wetlands in summertime when oxygen levels drop below 2.3 mg/L (Ostdiek and Nardone 1959, Crawford 1974, Morrow 1980), and also in partially iced-over waters during winter. The Central mudminnow *Umbra limi* breathes air from gas bubbles under ice for oxygen uptake (Magnuson *et al.* 1983), and blackfish may use a similar source of oxygen during winter under ice-covered lakes (personal observation).

Blackfish are also capable of living in shallow sphagnum ponds “where there is water [barely] enough to wet the skin of a fish” (Jordan and Evermann 1896).

Most blackfish overwinter in deeper reaches of ponds and lakes that do not freeze to the bottom (Reynolds 1997, Gudkov 1998). However, blackfish may be one of only two fish species that can survive in shallow ponds that freeze solid during winter; the other fish is the Crucian carp (*Carassius carassius*) of northern Europe (Ultsch 1989). Mud burial is suggested as a possible survival mechanism, although mud can clog gills and become highly anoxic; few fish can survive anoxia for extended periods, but 1 mg/L oxygen close to 0° C may be tolerated (Ultsch 1989). Mucus as a freeze protectant has also been suggested (Shaposhnikova 1960; Hargens 1973). One anecdote details blackfish harvested in winter by rural villagers who dug them out of rooted vegetation in side channels where they were encased in mucous cocoons. The water level had dropped after ice formation, and the blackfish habitat was exposed to air (J. Reynolds, University of Alaska Fairbanks, personal communication).

Blackfish predators include loons (*Galvia spp*.), mink (*Mustela vison*), river otter (*Lontra canadensis*), red fox (*Vulpes vulpes*), burbot (*Lota lota*), and sheefish (*Stenodus nelma*), as well as northern pike (Armstrong 2007). Stomach contents of 30 Lower Fire Lake (Anchorage Borough) adult pike sampled by the Alaska Department of Fish and Game (ADF&G) contained primarily blackfish.

Project Goals

Management actions have been taken to reduce or eliminate non-native blackfish populations in southcentral Alaska, in part due to the assumption that these introduced populations represent a significant risk to the viability of native fishes and natural resources. Here I explore the implicit hypothesis that introduced blackfish populations pose a measureable risk to the ecology of the invaded waterbodies and native fishes of southcentral Alaska. Specifically, I predict that fish constitute a major prey component in blackfish diets, and that blackfish diets overlap greatly with those of native fishes and stocked sportfish. Additionally, as most successful fish invaders show low dietary specialization and a high capacity to utilize available resources (Moyle and Light 1996a, 1996b, Marchetti *et al*. 2004, Gido and Franssen 2007), I predict that blackfish stomach contents vary significantly by waterbody, season, sex, and body size.

A husbandry manual to keep live blackfish is included (Appendix C). Captive blackfish may be useful to researchers and educators to better understand their behavior, development, physiology, and ecology.

Materials and Methods

Study Sites

Three freshwater habitats—a wetland, stream, and lake—were selected within the Cook Inlet Basin of southcentral Alaska, based on year-round presence of blackfish (Fig. 3). Duck Hunter's Pond and surrounding wetland (61.53920° N, 149.25460° W) within the Matanuska-Susitna Valley lowlands consist of a marsh, constructed rectangular pool, and narrow drainage ditch containing a large blackfish population. Blackfish are usually the only fish present in the wetland. The water in this pond is shallow, weedy, and hypoxic during all four seasons.

Rabbit Slough (61.53750° N, 149.25460° W) is a stream 0.15 km south of Duck Hunter's Pond, separated from the pond by a road, and within the Palmer Hayflats State Game Refuge. The stream drains into the Knik Arm of upper Cook Inlet and is characterized by slow-flowing, tannic-colored water with abundant overhanging vegetation. Soft silty benthos supports rooted macrophytes with some floating macrophytes as well. A coho salmon (*Oncorhynchus kisutch*) nursery and also a popular coho fishery, Rabbit Slough is home to other native fishes including Dolly Varden char (*Salvelinus malma*), threespine stickleback, ninespine stickleback (*Pungitius pungitius*), slimy sculpin (*Cottus cognatus*), and occasional stray sockeye salmon (*Oncorhynchus nerka*) and Chinook salmon (*Oncorhynchus tshawytscha*).

DeLong Lake (61.16390° N, 149.95550° W) in Anchorage is an 8-ha lake with mean depth of 4 m and maximum depth of 7 m. Invasive waterweed (*Elodea canadensis*) forms dense stands of long-stranded rooted macrophytes. This urban lake is a popular sport fishery stocked annually with hatchery rainbow trout and Chinook salmon. Catchables usually average 18 cm total length (TL) or longer; in 2010, 15,000 fingerling rainbow trout (mean TL 6.6 cm) were stocked (ADF&G 2011). Some local residents who consider blackfish to be a prized delicacy harvest them through the ice in DeLong Lake during late winter (personal observation).

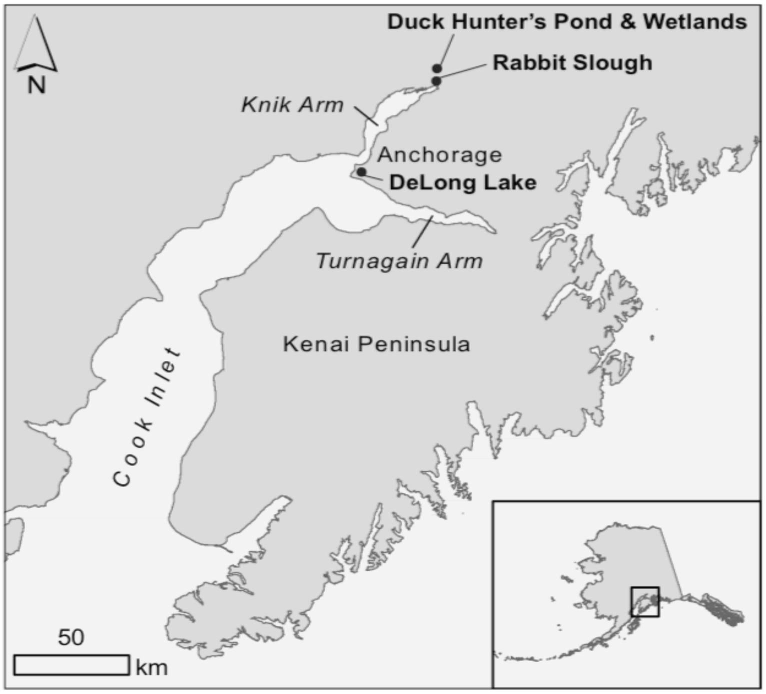

Figure 3. Map of study sites in the Cook Inlet Basin, Alaska.

Sampling Methodology

Once per month during a 12-month period, blackfish were captured from each location using 0.64 cm and 0.32 cm mesh unbaited minnow traps. Optimal trap-soaking times were three hours or less to avoid digestion of stomach contents while fish were in the traps; however, soaking times were increased if insufficient numbers of fish were being trapped for stomach contents analyses. Blackfish were euthanized with an overdose of pH-neutral MS-222 anesthetic then blotted and wet-weighed to the nearest 0.1 g. Fish were measured with digital calipers to the nearest 1.0 mm for TL and standard length (SL) and then injected through the mouth with buffered 37% formaldehyde solution to halt digestion. (Regurgitation during capture and lavaging of stomach contents during injection were rarely observed.) Whole specimens were placed into labeled teabags and fixed in buffered 10% formalin for 3 weeks prior to rinsing with water; they were then transferred to 70% ethanol.

Gastrointestinal tracts were dissected, and esophageal and stomach contents of each fish washed with 70% ethanol into a Petri dish. Prey items protruding into the mouth were also included. Prey organisms were viewed under a dissecting microscope, sorted, identified to an appropriate taxon (McCafferty 1998, Thorp and Covich 2001, Merritt *et al*. 2008), and counted. Mean weight for each prey type was obtained by drying and weighing a representative number of organisms. Prey were loaded into 3.5 × 5 mm or 4 × 6 mm pre-weighed pressed tin capsules, dried at 60° C in a drying oven, and weighed to the nearest 0.001mg on a Sartorius microscale. Larger prey were placed in Petri dishes, dried, and weighed to the nearest 0.001g on an analytical balance. A subset of each prey type was then used to compute mean weight for that prey category. Digested prey without identifiable parts were excluded. Intestinal contents were removed for identification but not counted.

Stomach Contents Analysis

To obtain a measure of overall importance of each prey category, I used the index of relative importance (IRI)

$$IRI = (\%N + \%M) * (\%F)$$

where number (N) equals the actual count of individual prey items and highlights the importance of small prey such as zooplankton; mass (M) equals the dry mass of prey items and emphasizes large, bulky prey; and frequency (F) equals the number of stomachs containing a specific food organism (Pinkas *et al*. 1971, Cailliet *et al*. 1986), . IRI values were computed for the following eight groupings: combined sites and seasons, combined sites by season, combined seasons by site, each site by season, males and females by combined sites and seasons, and size classes by combined sites and seasons. Fish were grouped into one size class up to 65 mm SL, five 10-mm size classes from 65–115 mm SL, and one size class from 115–148 mm SL.

Statistical Analysis

All statistical analyses were performed using SPSS v.22. A General Linear Model MANCOVA was used to test for differences in diet among waterbodies, among seasons, and by sex. For the dependent variables, diet value consisted of prey masses of nine prey categories equal to or greater than 1% IRI for combined sites and seasons. Covariates were standard length and trapping hours. Additional MANCOVAs were performed to test for seasonal differences in diet within each site.

Size differences between sexes were analyzed by performing a two-sample Student's *t*-test assuming equal variances using a pooled estimate of the variance. A binary logistic regression was also performed to analyze presence of fish in diet, using sex and size class as predictor variables.

Results

Trapping Times and Specimen Counts

Trapping times ranged from 2.5–13.5 hrs with a mean of 5.7 hrs; processing times ranged from 0.5–5.5 hrs with a mean of 1.9 hrs. Total trapping plus processing times (time from trap placed in water to specimen placed into formalin) ranged from 3.5–14.5 hrs with a mean of 7.6 hrs (Figs. 4 and 5). Trapping times in excess of 10 hours occurred overnight during summer in the lake and stream. Overall prey consumption by trapping hours was not significant (F (9, 244) = 1.779, $p = 0.073$).

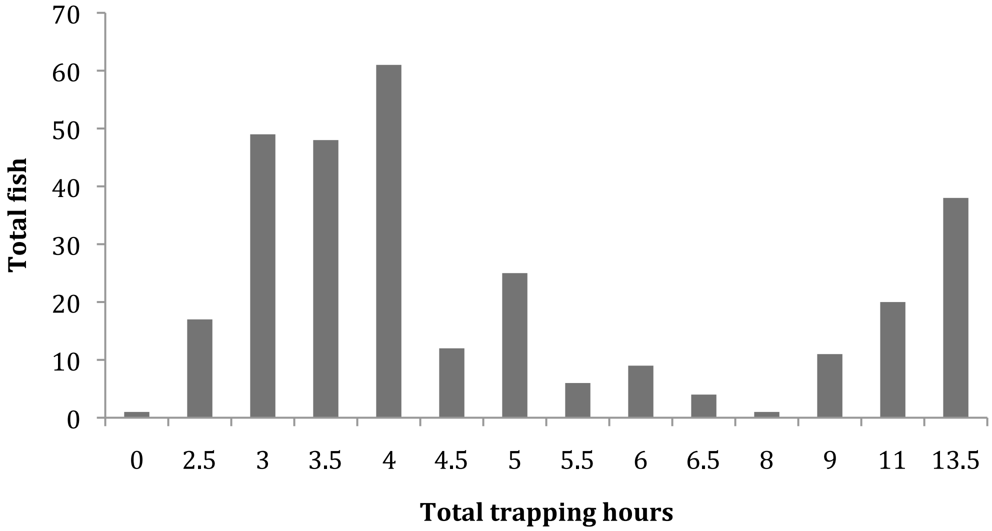

Figure 4. Total trapping hours distribution of diet study fish (*n* = 302).

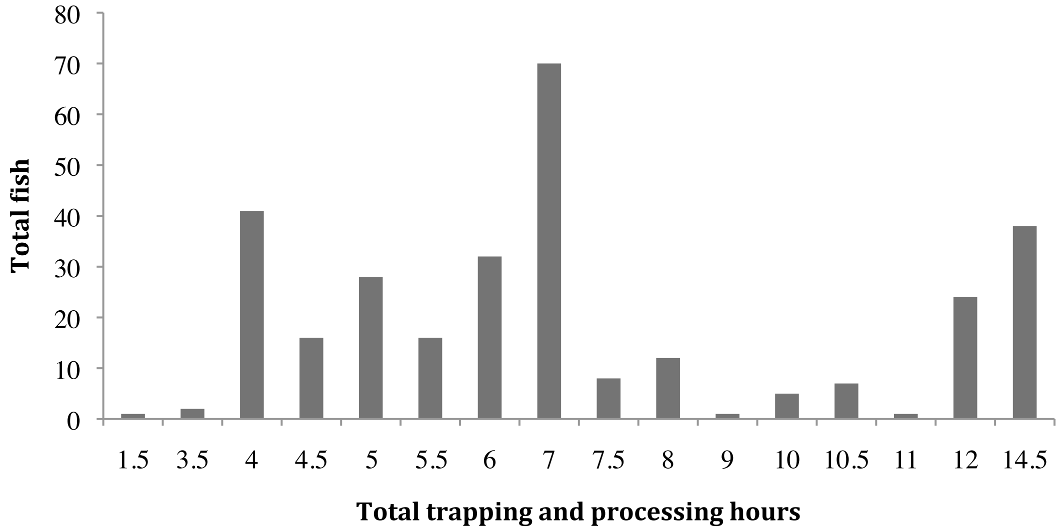

Figure 5. Total trapping hours plus processing hours distribution of diet study fish (*n* = 302). Processing time equals the time from the removal of live fish from trap until fish is euthanized, measured, and injected with formalin.

From a total of 470 blackfish collected and fixed in formalin, 302 fish were dissected for stomach contents analysis: 84 from the lake, 104 from the stream, and 114 from the wetland (Table 1). In springtime, only wetland fish were captured and analyzed due to unsafe ice conditions on DeLong Lake and Rabbit Slough. Overall, 78% of dissected blackfish were trapped during daytime compared to 22% trapped overnight, in summer, from the lake and stream.

Table 1. Total fish dissected for diet study. Bold numbers represent totals. Numbers in parentheses are fish trapped during nighttime. Note that no fish were collected in spring from the lake or stream.

	Lake	Stream	Wetland	**TOTAL**
Spring	–	–	**22**	**22**
APR	–	–	8	8
MAY	–	–	14	14
Summer	**27**	**50**	**29**	**106**
JUN	2	6 (20)	17	45
JUL	4	(17)	0	21
AUG	(21)	(7)	12	40
Autumn	**29**	**20**	**18**	**67**
SEP	5	9	10	24
OCT	24	11	8	43
Winter	**28**	**34**	**45**	**107**
NOV	0	28	20	48
DEC	16	4	14	34
JAN	6	0	0	6
FEB	5	1	1	7
MAR	1	1	10	12
TOTAL	**84**	**104**	**114**	**302**

Body Size Comparisons

Size comparisons of males and females dissected for the diet study (excluding three specimens of unknown sex) show that males are generally larger than females (Fig. 6; Table 2). The mean SL of males (mean = 98.90, *SD* = 15.51, *n* = 145) was significantly greater than that of females (mean = 91.67, *SD* = 15.44, *n* = 154; *t* (297) = 7.23, $p < 0.001$). Seventy-five percent of all fish were in the 75–115 mm SL size class (Fig. 7).

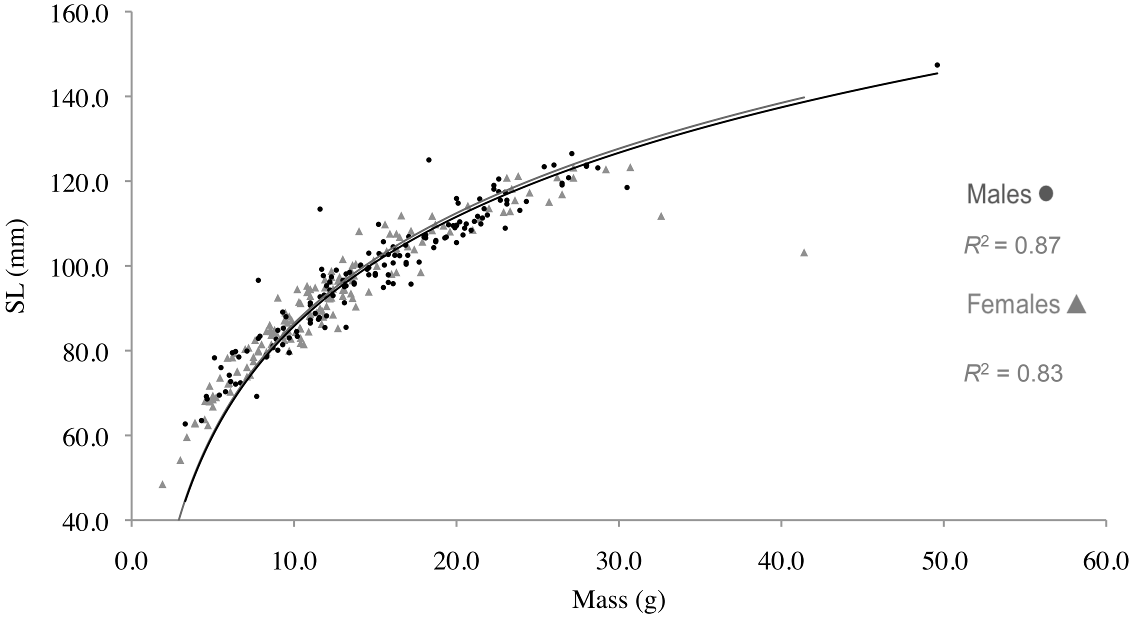

Figure 6. Standard length (SL) vs. mass of diet study fish. Dark circles represent males (*n* = 145); light triangles represent females (*n* = 154).

Table 2. Size comparisons for males and females used in diet study.

Males	Mean	SE	Range
Mass (g)	15.55	0.57	3.3 - 49.6
SL (mm)	98.90	1.29	62.7 - 147.4
TL (mm)	119.47	1.50	73.3 - 178.1
Females	**Mean**	**SE**	**Range**
Mass (g)	12.71	0.52	1.9 - 30.7
SL (mm)	91.67	1.24	48.5 - 123.3
TL (mm)	110.51	1.48	60.8 - 149.1

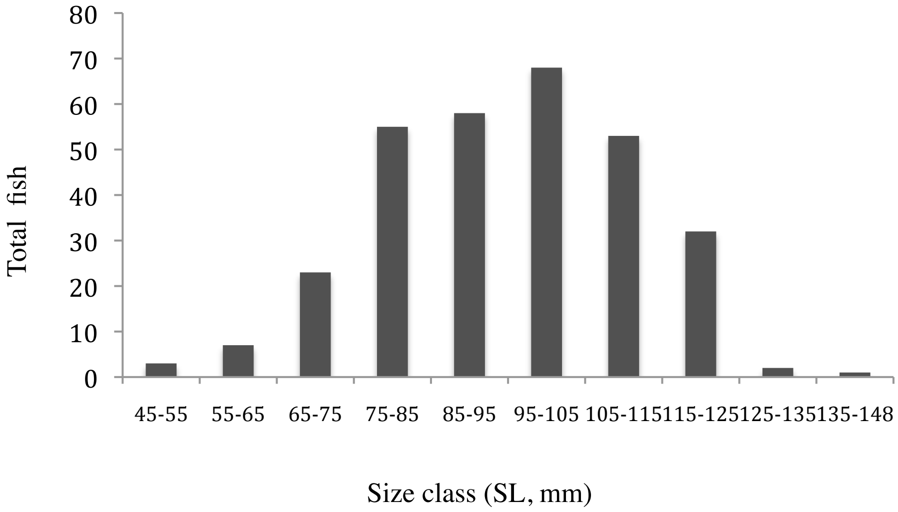

Figure 7. Size class distribution of diet study fish ($n = 302$).

Prey Categories

Gut contents of Cook Inlet Basin blackfish from the three study sites belonged to 20 taxonomic groups distributed among five animal phyla (mollusks, annelids, arthropods, bryozoans, and chordates) and one plant division (Table 3; taxa in bold indicate categories used for the analysis). For the stomach contents analysis, Class Insecta was differentiated as Insecta adults, Diptera larvae, Trichoptera larvae, Odonata larvae, and Ephemeroptera larvae. Order Diptera was subdivided into six families in Table 3 in order to highlight the diversity of this major prey category, although family groupings were not included in the stomach contents analysis. Gut contents from Order Coleoptera included both larvae and adults. Bryozoa/Plumatellida from DeLong Lake consisted of over-wintering cysts (statoblasts). Angiospermae in blackfish gut contents were represented by seeds of unknown plants. Occasional plant stem and leaf tissue found in the guts of blackfish were excluded from analysis because it was assumed they were accidentally swallowed and non-digestible.

Table 3. All prey categories identified in gastrointestinal tracts. Habitat zones are generalized (McCafferty 1998). E = epiphytic; B = benthic; NB = near benthic; O = open water; S = surface; NS = near surface.

Prey categories	Common name	Habitat
Ph. Mollusca		
Cl. **Gastropoda**	snails	E, B
Cl. **Bivalvia**	clams	B
Ph. Annelida		
Cl. **Hirudinea**	leeches	E, B
Ph. Arthropoda		
Cl. Arachnida		
Hydracarina	water mites	E
Or. **Araneae**	spiders	S
Subph. Crustacea		
Cl. **Ostracoda**	seed shrimp	E, B
Cl. **Copepoda**		B, O
Cl. Phyllopoda		
Subor. **Cladocera**	water fleas	O, E
Cl. Malocostraca		
Subor. **Gammaridea**	scuds	E, B
Subph. Hexapoda		
Or. **Collembola**	springtails	S
Cl. **Insecta (adults)**		S
Or. **Diptera (larvae)**	true flies	E, B
F. Ceratopogonidae	biting midges	
F. Chaoboridae	phantom midges	
F. Chironomidae	bloodworms	
F. Psychodidae	moth flies	
F. Syrphidae	rat-tailed maggots	
F. Tipulidae	crane flies	
Or. **Trichoptera (larvae)**	caddisflies	E, B
Or. **Coleoptera**	beetles	E, B, NS
Or. Hemiptera	true bugs	
F. **Corixidae**	water boatmen	NB
Or. **Odonata (larvae)**	dragonflies	E
Or. **Ephemeroptera (larvae)**	mayflies	E
Ph. Bryozoa		
Or. **Plumatellida (statoblasts)**	moss animals	E, B
Ph. Chordata		
Infrcl. **Teleostei**	bony fishes	
Oncorhynchus kisutch	Coho salmon	O
Dallia pectoralis	Alaska blackfish	E, B
Gasterosteus aculeatus	threespine stickleback	O
Pungitius pungitius	ninespine stickleback	O
Div. **Angiospermae (seeds)**	flowering plants	E, B

Diet Diversity

Consistent with the prediction of a high diet diversity, blackfish in these populations typically had many prey types in their guts (gastrointestinal tracts). The number of different prey types, based on 20 prey categories, found in each nonempty gut (esophagus + stomach + intestines) ranged from 1–10 (Fig. 8). Most lake blackfish guts contained three prey types with 92% total guts having 1–6 prey groups. Stream fish also had three prey types in their guts, although 78% had 2–5 prey categories. Finally, wetland guts contained the greatest diversity of prey; most wetland fish consumed 4 prey types, and overall 84% of wetland blackfish consumed 3–8 different prey taxa.

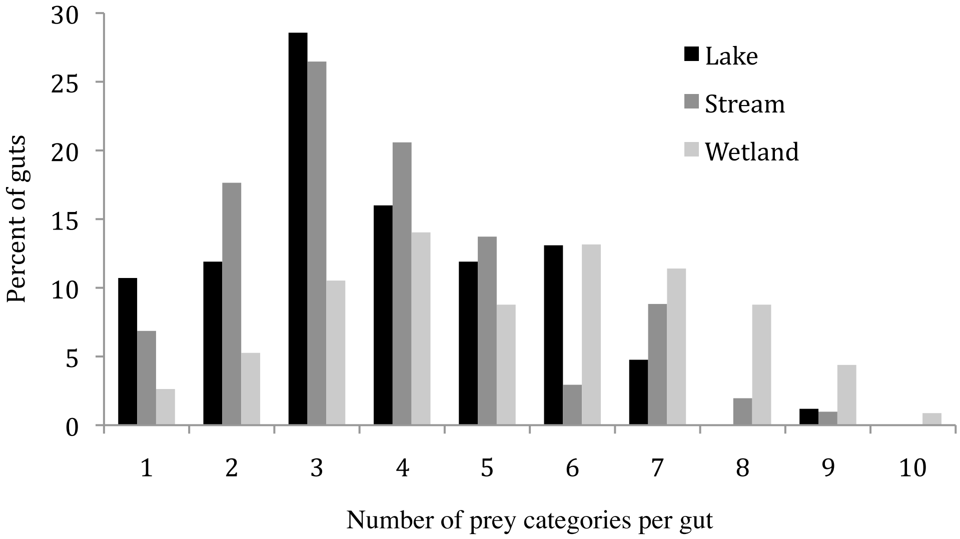

Figure 8. Diet diversity histogram. For each site, the percentage of guts containing 1–10 prey categories is given.

Statistical Analysis

Table 4 summarizes multivariate effects based on three factors (waterbody, season, and sex), two covariates (SL, trapping hours), and nine response variables (nine prey categories whose % IRI ≥ 1; see Table 5). Between-subjects effects are displayed in Appendix B.1. Waterbody and season were significant factors, as was the interaction between them. Fish size and sex were not significant, and neither was trapping hours.

Table 4. Multivariate effects. The MANCOVA is based on three factors (waterbody, season, and sex), two covariates (SL, trapping hours), and nine response variables (nine prey categories whose % IRI ≥ 1). SL = standard length, *ns* = not significant.

Variable	Pillai's Trace	*F*	Hypothesis *df*	Error *df*	*p*
SL	0.063	1.82	9	244	*ns*
Trapping hrs.	0.062	1.78	9	244	*ns*
Site	0.287	4.56	18	490	< 0.001
Season	0.231	3.56	18	490	< 0.001
Sex	0.061	1.76	9	244	*ns*
Site × season	0.413	3.16	36	988	< 0.001

Stomach Contents Analysis

<u>Stomach Contents by Combined Sites and Seasons</u>

An index of relative importance diagram (Fig. 9) was constructed that portrays prey categories ≥ 3% IRI as rectangles whose areas are derived from Pinkas' *et al.* (1971) formula, IRI = (%N + %M) * (%F). All prey values, including those for minor prey not shown in the diagram, are also displayed in Table 5. For combined waterbodies and seasons, Gastropoda were by far the dominant prey group (51% IRI). Ostracoda contributed 28% of the percent IRI, while all other prey taxa recorded 7% IRI or less. Twelve categories recorded only minor importance (%IRI < 0.5%). The smaller ostracods were the primary prey in terms of total number, while larger gastropods and teleosts were the two most important prey in terms of biomass. Dipterans were found most frequently in guts (62% frequency), followed by gastropods (46% frequency). Overall, six prey taxa were consumed by at least 25% of all blackfish: dipteran larvae, gastropods, copepods, trichopteran larvae, ostracods, and bivalves. Plant seeds (Angiospermae) were found in 14% of all guts. Contrary to my prediction that fish represent a major component of blackfish diet, fish as prey ranked 4th in importance by percent IRI, while frequency of fish consumption was less than 10%.

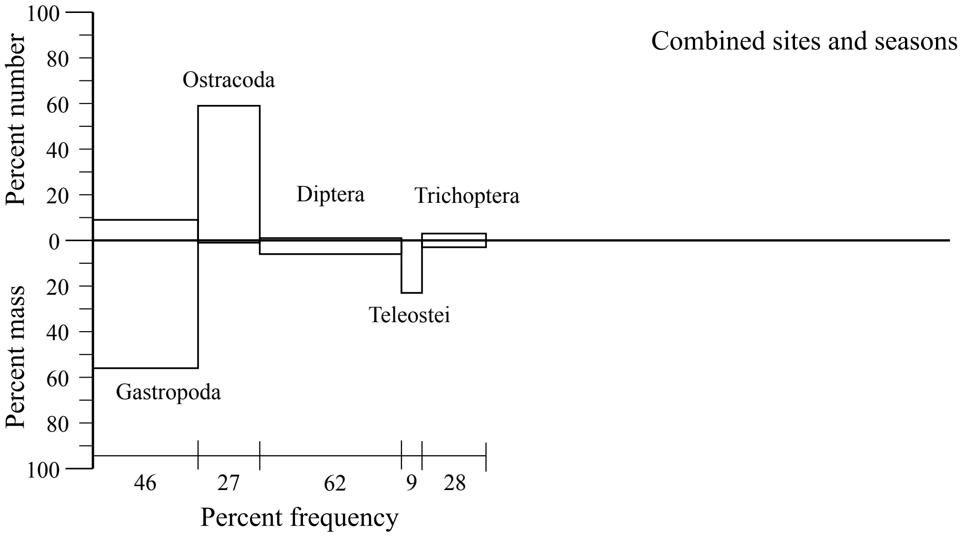

Figure 9. Prey IRI diagram for combined sites and seasons. Only major prey categories ≥3% IRI are shown. Frequency of occurrence axis begins at zero for each prey category.

Table 5. Prey values for combined sites and seasons ($n = 302$). Major and minor prey categories are given in descending order by percent IRI. Grey-colored rows are prey categories shown in IRI diagram (Fig. 9). Diptera, Odonata, and Trichoptera, and Ephemeroptera represent larvae.

Prey categories	Total no.	% no.	Total mass (mg)	% mass	% freq	IRI	% IRI
Gastropoda	1307	8.7	6697.817	56.2	46.4	3009	50.5
Ostracoda	8921	59.1	142.214	1.2	27.5	1658	27.8
Diptera	187	1.2	657.617	5.5	61.9	419	7.0
Teleostei	35	0.2	2729.331	22.9	9.3	216	3.6
Trichoptera	512	3.4	354.070	3.0	28.1	179	3.0
Bivalvia	262	1.7	495.639	4.2	25.2	148	2.5
Copepoda	643	4.3	10.288	0.1	30.5	132	2.2
Cladocera	1123	7.4	23.583	0.2	13.2	101	1.7
Angiospermae	172	1.1	172.860	1.5	14.2	37	0.6
Odonata	95	0.6	207.100	1.7	10.6	25	0.4
Coleoptera	37	0.2	237.826	2.0	7.6	17	0.0
Plumatellida	429	2.8	38.610	0.3	3.6	12	0.0
Corixidae	30	0.2	108.330	0.9	4.0	4	0.0
Gammaridae	28	0.2	12.852	0.1	5.3	2	0.0
Insecta adult	6	0.0	11.005	0.1	1.7	0	0.0
Araneae	6	0.0	7.533	0.1	2.0	0	0.0
Hydracarina	10	0.1	0.560	0.0	2.3	0	0.0
Ephemeroptera	6	0.0	1.304	0.0	1.7	0	0.0
Hirudinea	2	0.0	0.998	0.0	0.7	0	0.0
Unknowns	2	0.0	0.337	0.0	0.7	0	0.0
Collembola	1	0.0	0.052	0.0	0.3	0	0.0

Stomach Contents by Site

Differential prey consumption by waterbody for seasons pooled was highly significant (Pillai's Trace = 0.287, F (18, 490) = 4.56, $p < 0.001$). A different prey taxon dominated gut contents for each site: Diptera (66% IRI) in lake fish, Gastropoda (78% IRI) in stream fish, and Ostracoda (59% IRI) in wetland fish (Fig. 10–12; Tables 6–8). All other prey values at each site were less than 20% IRI. Teleosts contributed at least 60% of the percent biomass in gut contents from lake fish, while gastropods contributed

70% of overall biomass gut contents for stream fish. No single taxon dominated by percent biomass for wetland fish. Dipteran larvae were the most frequently consumed prey by lake fish, compared to gastropods and dipteran larvae for stream fish. Wetland blackfish had the greatest variety of prey in their guts, consuming three taxa at 50% or greater frequency and three additional taxa at frequencies of 24%–35%. Fish as prey ranked third in importance for both lake and stream blackfish but were excluded from prey rankings above 3% for wetland blackfish.

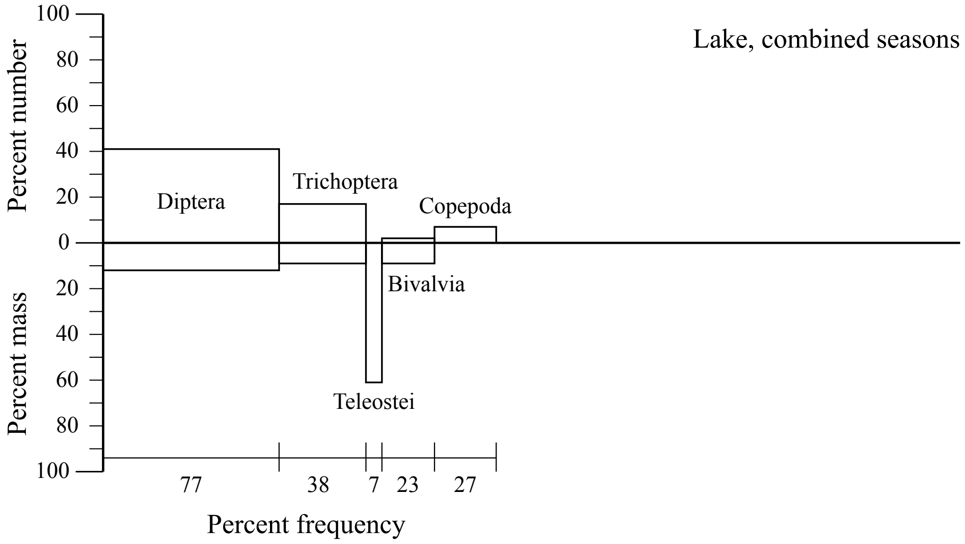

Figure 10. Prey IRI diagram for lake site, combined seasons. (Springtime is excluded.) Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

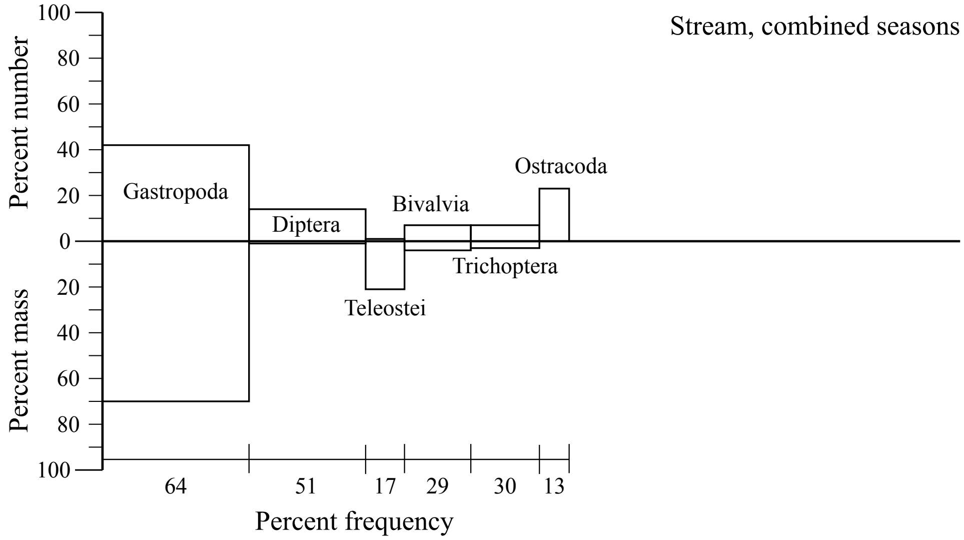

Figure 11. Prey IRI diagram for stream site, combined seasons. (Springtime is excluded.) Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

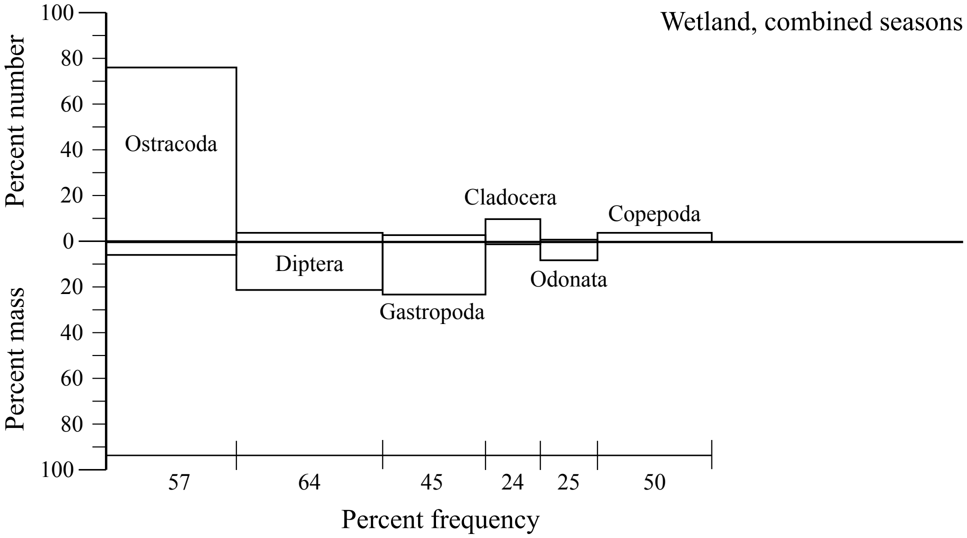

Figure 12. Prey IRI diagram for wetland site, combined seasons. (Springtime is excluded.) Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 6. Prey values for the lake site, combined seasons (spring exluded). Grey-colored rows are prey categories shown in IRI diagram (Fig. 10).

Lake, combined seasons **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Diptera	721	41	111.295	12	77	4040	66
Trichoptera	299	17	84.153	9	38	962	16
Teleostei	7	0	583.386	61	7	419	7
Bivalvia	43	2	81.657	9	23	249	4
Copepoda	130	7	2.080	0	27	206	3
Gastropoda	44	2	13.449	1	31	119	2
Plumatellida	429	0	38.610	4	13	53	1
Angiospermae	18	1	18.090	2	11	33	1
Ostracoda	42	2	0.630	0	13	30	0
Cladocera	18	1	0.378	0	9	10	0
Gammaridae	14	1	6.426	1	6	8	0
Odonata	6	0	13.080	1	5	8	0
Insecta adult	1	0	2.623	0	1	0	0
Hirudinea	1	0	0.607	0	1	0	0
Hydracarina	1	0	0.056	0	1	0	0

Table 7. Prey values for the stream site, combined seasons (spring exluded). Grey-colored rows are prey categories shown in IRI diagram (Fig. 11).

Stream, combined seasons **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	960	42	6171.633	70	64	7260	78
Diptera	317	14	77.337	1	51	755	8
Teleostei	23	1	1863.378	21	17	386	4
Bivalvia	166	7	315.234	4	29	314	3
Trichoptera	167	7	223.414	3	30	295	3
Ostracoda	522	23	7.867	0	13	288	3
Copepoda	61	3	0.976	0	15	41	0
Corixidae	16	1	57.776	1	8	10	0
Angiospermae	11	0	11.055	0	11	6	0
Cladocera	13	1	0.273	0	7	4	0
Coleoptera	5	0	17.581	0	3	1	0
Gammaridae	5	0	2.295	0	3	1	0
Odonata	2	0	4.36	0	2	0	0
Hydracarina	2	0	0.112	0	2	0	0
Insecta adult	2	0	0.513	0	1	0	0
Ephemeroptera	1	0	0.674	0	1	0	0
Hirudinea	1	0	0.391	0	1	0	0
Collembola	1	0	0.052	0	1	0	0

Table 8. Prey values for the wetland site, combined seasons (spring exluded). Grey-colored rows are prey categories shown in IRI diagram (Fig. 12).

Wetland, combined seasons **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	7029	75	106.397	8	52	4345	59
Copepoda	443	5	7.088	1	50	264	11
Cladocera	1092	12	22.932	2	27	365	11
Diptera	356	4	137.580	11	53	777	5
Trichoptera	30	0	22.936	2	17	37	5
Odonata	83	1	180.940	14	24	361	4
Gastropoda	144	2	268.975	21	35	787	3
Bivalvia	25	0	45.576	4	15	58	1
Teleostei	3	0	225.432	18	2	39	1
Angiospermae	134	1	134.670	11	18	222	1
Hydracarina	3	0	0.168	0	3	0	0
Araneae	3	0	3.295	0	3	1	0
Gammaridae	8	0	3.672	0	8	3	0
Insecta adult	3	0	7.869	1	3	2	0
Coleoptera	14	0	91.669	7	10	72	0
Corixidae	4	0	14.444	1	1	1	0
Ephemeroptera	5	0	0.630	0	4	0	0
Unknowns	2	0	0.337	0	2	0	0

Stomach Contents by Season

Prey consumption differed by season (spring excluded) for waterbodies pooled (Pillai's Trace = 0.231, F (18, 490) = 3.56, $p < 0.001$) (Fig. 13–15; Tables 9–11). During summer, gastropods were the single major prey (69%IRI), while all other groups contributed less than 10%IRI. Gastropods also dominated in summer by biomass (79%), followed by teleosts (10% mass). More than half of all guts in summer contained gastropods and dipterans, and small ostracods and cladocerans outnumbered larger prey.

Dominant prey groups in autumn consisted of gastropods and ostracods, which contributed 24%–48% of the percent IRI values. Gastropods were the most important prey in terms of biomass (61%). At least 45% of all guts in autumn contained dipterans, gastropods, trichopterans, and ostracods.

Prey in winter switched to ostracods (39%IRI) followed by dipterans (24%IRI) and teleosts (19% IRI). Fish were the most dominant winter prey by biomass (72%), although their total count (13) was small compared to ostracods (4,452). The most frequently consumed winter prey were dipteran larvae and copepods.

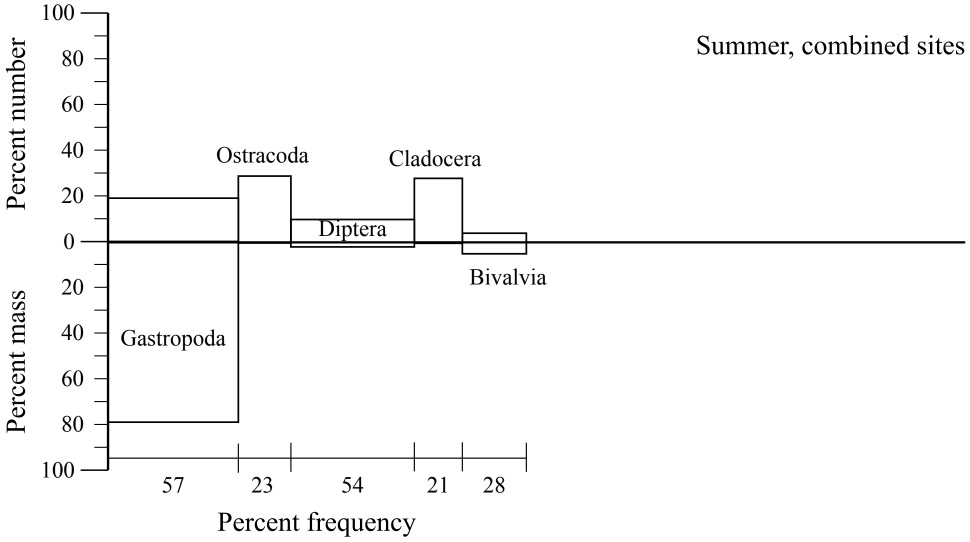

Figure 13. Prey IRI diagram for summer, combined sites. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

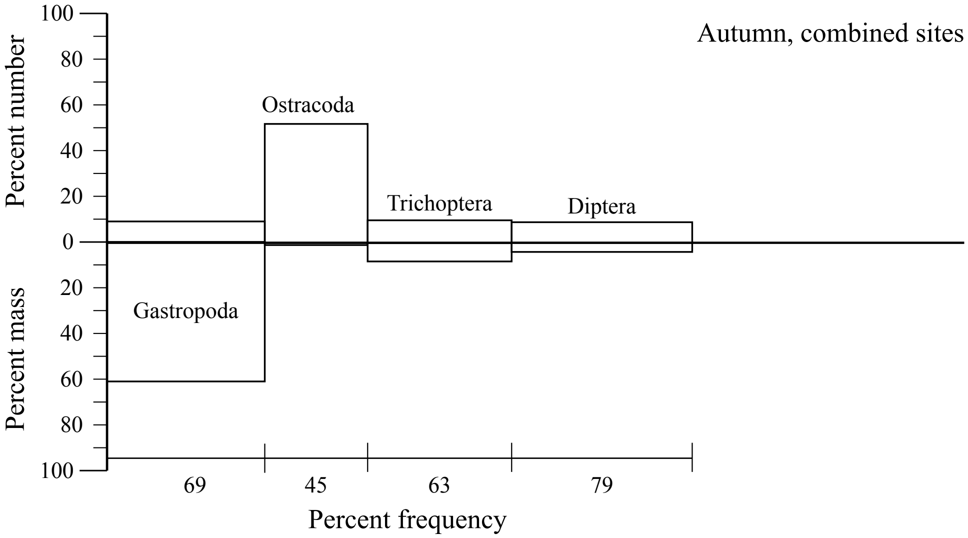

Figure 14. Prey IRI diagram for autumn, combined sites. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

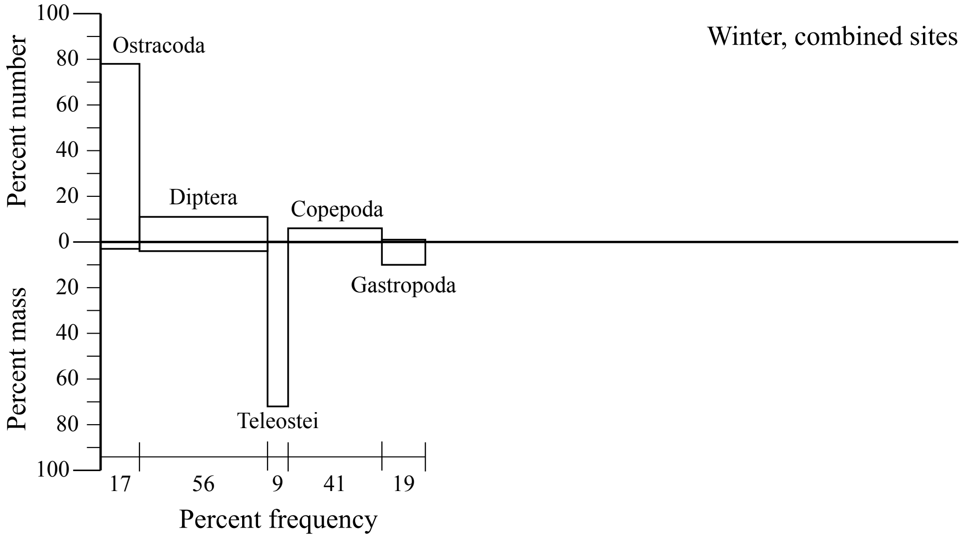

Figure 15. Prey IRI diagram for winter, combined sites. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 9. Prey values for summer, combined sites. Grey-colored rows are prey categories shown in IRI diagram (Fig. 13).

Summer, combined sites **Prey categories**	**Total No.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	723	19	4393.520	79	57	5537	69
Ostracoda	1087	29	16.342	0	23	658	8
Diptera	384	10	98.763	2	54	641	8
Cladocera	1071	28	22.491	0	21	596	7
Bivalvia	150	4	284.850	5	28	257	3
Teleostei	16	0	563.021	10	11	119	1
Trichoptera	92	2	53.543	1	24	80	1
Copepoda	185	5	2.960	0	15	75	1
Angiospermae	29	1	29.145	1	15	19	0
Coleoptera	10	0	58.786	1	7	9	0
Odonata	13	0	28.340	1	8	6	0
Insecta adult	5	0	10.527	0	4	1	0
Corixidae	5	0	18.055	0	2	1	0
Hydracarina	3	0	0.168	0	3	0	0
Ephemeroptera	2	0	0.856	0	2	0	0
Hirudinea	1	0	0.607	0	1	0	0
Gammaridae	1	0	0.459	0	1	0	0
Araneae	1	0	0.203	0	1	0	0
Plumatellida	1	0	0.090	0	1	0	0
Unknowns	1	0	0.060	0	1	0	0

Table 10. Prey values for autumn, combined sites. Grey-colored rows are prey categories shown in IRI diagram (Fig. 14).

Autumn, combined sites **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	371	9	1828.234	61	69	4820	48
Ostracoda	2054	52	31.735	1	45	2370	24
Trichoptera	380	10	233.912	8	63	1089	11
Diptera	368	9	134.581	4	79	1089	11
Angiospermae	115	3	115.575	4	18	121	1
Plumatellida	420	11	37.800	1	9	106	1
Copepoda	95	2	1.520	0	39	95	1
Bivalvia	33	1	60.768	2	28	81	1
Teleostei	4	0	376.327	13	6	75	1
Odonata	35	1	76.300	3	18	61	1
Cladocera	43	1	0.903	0	21	23	0
Coleoptera	9	0	50.464	2	7	14	0
Corixidae	13	0	46.943	2	7	14	0
Gammaridae	13	0	5.967	0	13	7	0
Araneae	2	0	3.092	0	3	0	0
Insecta adult	1	0	0.478	0	1	0	0
Unknowns	1	0	0.277	0	1	0	0
Ephemeroptera	1	0	0.182	0	1	0	0
Hydracarina	1	0	0.056	0	1	0	0
Collembola	1	0	0.052	0	0	0	0

Table 11. Prey values for winter, combined sites. Grey-colored rows are prey categories shown in IRI diagram (Fig. 15).

Winter, combined sites							
Prey categories	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	4452	78	66.817	3	17	1363	39
Diptera	642	11	94.028	4	56	852	24
Teleostei	13	0	1732.848	72	9	677	19
Copepoda	354	6	5.664	0	41	266	8
Gastropoda	54	1	232.303	10	19	199	6
Bivalvia	51	1	96.849	4	14	69	2
Odonata	43	1	93.740	4	7	35	1
Trichoptera	24	0	43.048	2	12	27	1
Angiospermae	19	0	19.095	1	9	11	0
Gammaridae	13	0	5.967	0	5	2	0
Plumatellida	8	0	0.720	0	4	1	0
Corixidae	2	0	7.222	0	2	1	0
Cladocera	9	0	0.189	0	4	1	0
Ephemeroptera	3	0	0.266	0	2	0	0
Hydracarina	2	0	0.112	0	2	0	0
Hirudinea	1	0	0.391	0	1	0	0

Stomach Contents for Lake by Season

Gut contents of lake blackfish varied significantly by season (Pillai's Trace = 0.531, F (18, 138) = 2.769, $p < 0.001$) (Fig. 16–18, Tables 12–14). During summer, teleosts, trichopteran larvae, and dipteran larvae contributed at least 24% of the %IRI values, while trichopteran larvae were the most dominant prey during autumn (54%IRI). Bryozoans statoblasts ranked third in importance by %IRI during autumn. Dominant wintertime prey were dipteran larvae and copepods (50%IRI and 30%IRI respectively). By %mass, teleosts were most the important prey in summer and winter, while trichopterans, bryozoans, and dipterans contributed at least 13% of the biomass during autumn. Dipteran larvae were found in at least half of all guts during all seasons.

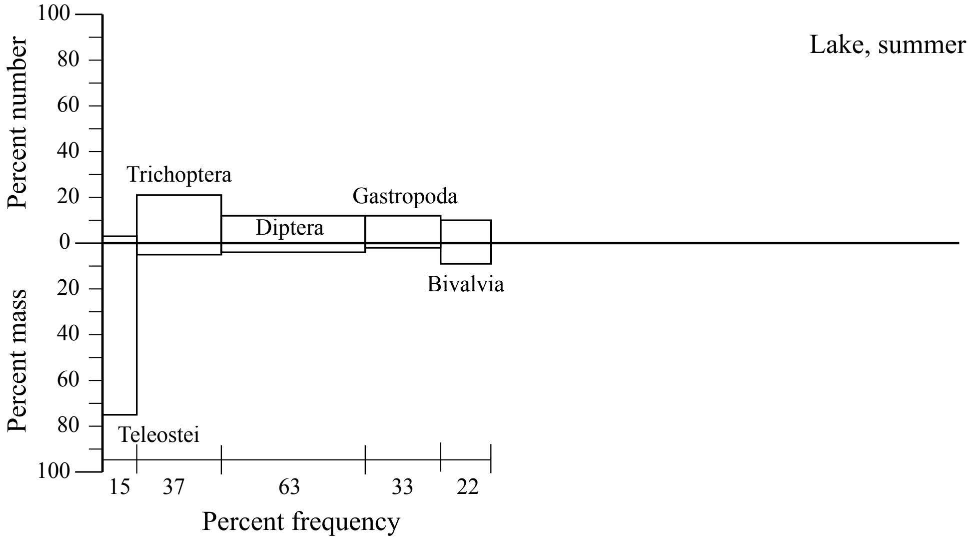

Figure 16. Prey IRI diagram for lake site, summer. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

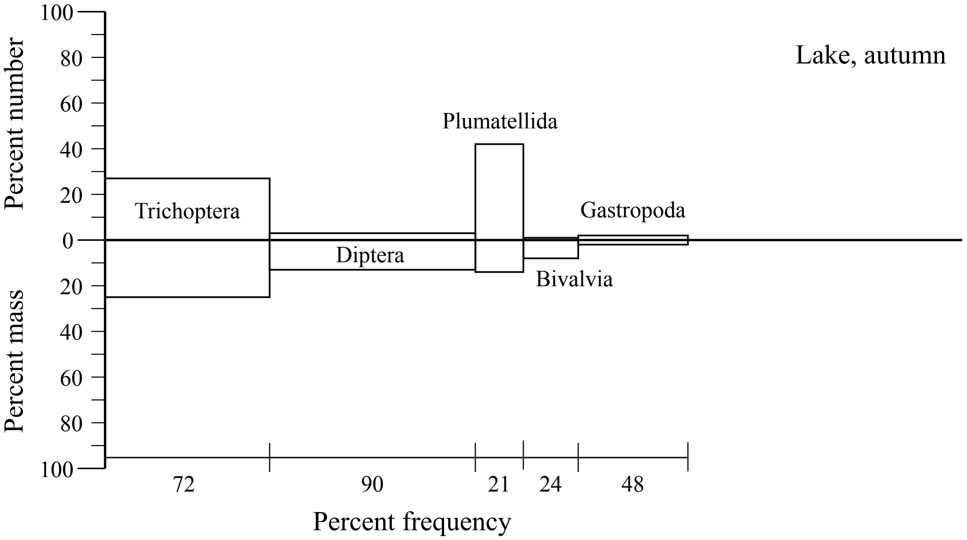

Figure 17. Prey IRI diagram for lake site, autumn. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

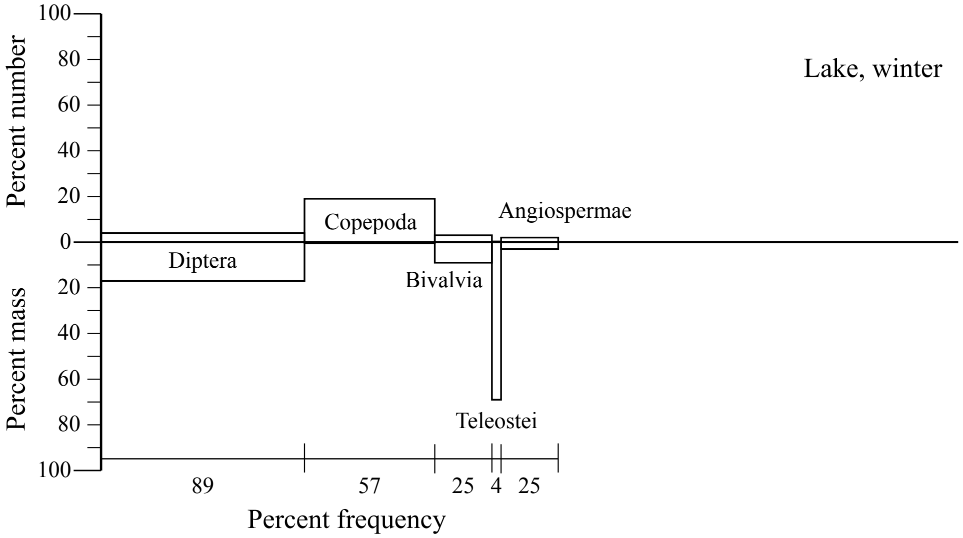

Figure 18. Prey IRI diagram for lake site, winter. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 12. Prey values for lake site, summer. Grey-colored rows are prey categories shown in IRI diagram (Fig. 16).

Lake, summer							
Prey categories	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Teleostei	4	3	219.870	75	15	1157	29
Trichoptera	31	21	14.041	5	37	959	24
Diptera	17	12	10.479	4	63	954	24
Gastropoda	18	12	5.634	2	33	473	12
Bivalvia	14	10	26.586	9	22	414	10
Angiospermae	5	3	5.025	2	4	19	0
Odonata	3	2	6.540	2	4	16	0
Cladocera	2	1	0.042	0	7	10	0
Insecta adult	1	1	2.623	1	4	6	0
Hirudinea	1	1	0.607	0	4	3	0
Plumatellida	1	1	0.090	0	4	3	0
Hydracarina	1	1	0.056	0	4	3	0
Copepoda	1	1	0.016	0	4	3	0

Table 13. Prey values for lake site, autumn. Grey-colored rows are prey categories shown in IRI diagram (Fig. 17).

Lake, autumn							
Prey categories	Total no.	% no.	Total Mass (mg)	% mass	% freq.	IRI	% IRI
Trichoptera	266	27	69.698	25	72	3755	54
Diptera	26	3	34.863	13	90	1366	19
Plumatellida	420	42	37.800	14	21	1152	16
Bivalvia	11	1	20.889	8	24	209	3
Gastropoda	20	2	5.947	2	48	201	3
Teleostei	1	0	96.516	35	3	121	2
Ostracoda	39	4	0.585	0	28	113	2
Cladocera	16	2	0.336	0	21	36	1
Copepoda	9	1	0.144	0	24	23	0
Odonata	2	0	4.360	2	7	12	0
Angiospermae	3	0	3.015	1	7	10	0
Gammaridae	4	0	1.836	1	7	7	0

Table 14. Prey values for lake site, winter. Grey-colored rows are prey categories shown in IRI diagram (Fig. 18).

Lake, winter Prey categories	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Diptera	25	4	65.953	17	89	1870	50
Copepoda	120	19	1.920	0	57	1122	30
Bivalvia	18	3	34.182	9	25	291	8
Teleostei	2	0	267.000	69	4	246	7
Angiospermae	10	2	10.050	3	25	104	3
Gammaridae	10	2	4.590	1	11	30	1
Plumatellida	8	1	0.720	0	14	21	1
Gastropoda	6	1	1.868	0	14	21	1
Ostracoda	3	0	0.045	0	11	5	0
Trichoptera	2	0	0.414	0	7	3	0
Odonata	1	0	2.180	1	4	3	0

Stomach Contents for Stream by Season

Gut contents of stream blackfish varied significantly by season (Pillai's Trace = 0.663, F (18, 174) = 4.793, $p < 0.001$). Gastropods were by far the dominant prey during summer and autumn, in terms of %IRI, %frequency, and %mass (Fig. 19–21; Tables 15–17). During winter, dipteran larvae contributed greater than 50% of the %IRI, %frequency, and also %number values. Teleosts contributed 23% of the %IRI values during winter, and in terms of biomass, were the most important prey. Prey diversity was greatest during winter; six taxa contributed at least 3% of the %IRI values for winter in the stream, compared to three taxa each during summer and autumn.

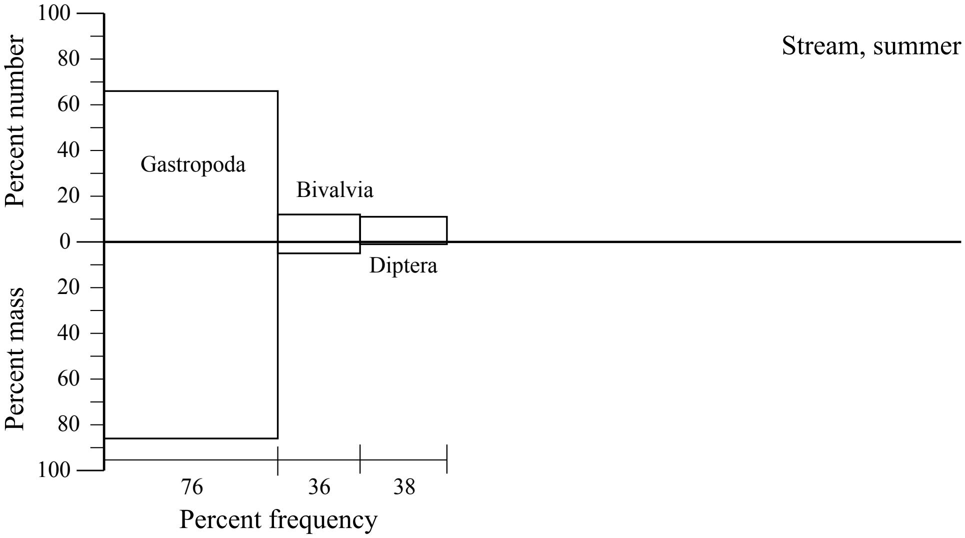

Figure 19. Prey IRI diagram for stream site, summer. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

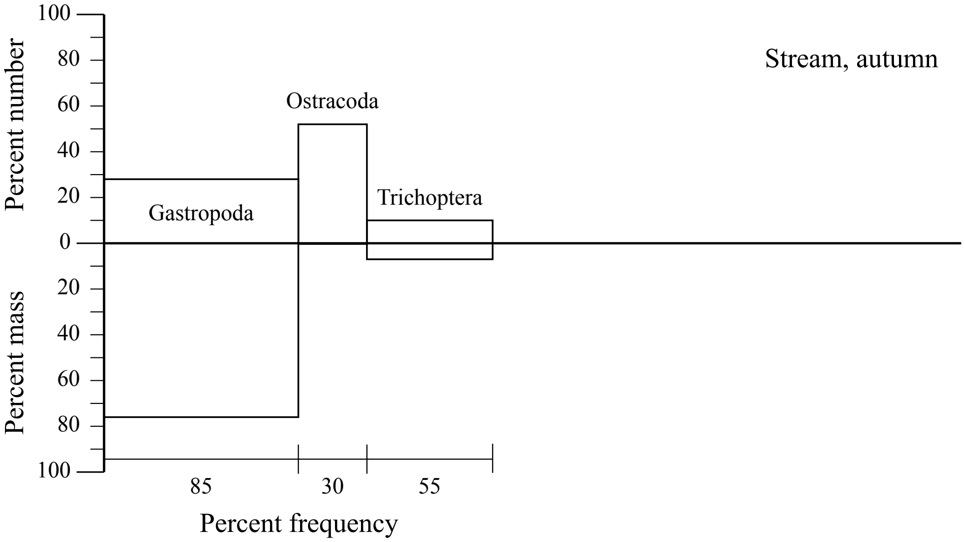

Figure 20. Prey IRI diagram for stream site, autumn. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

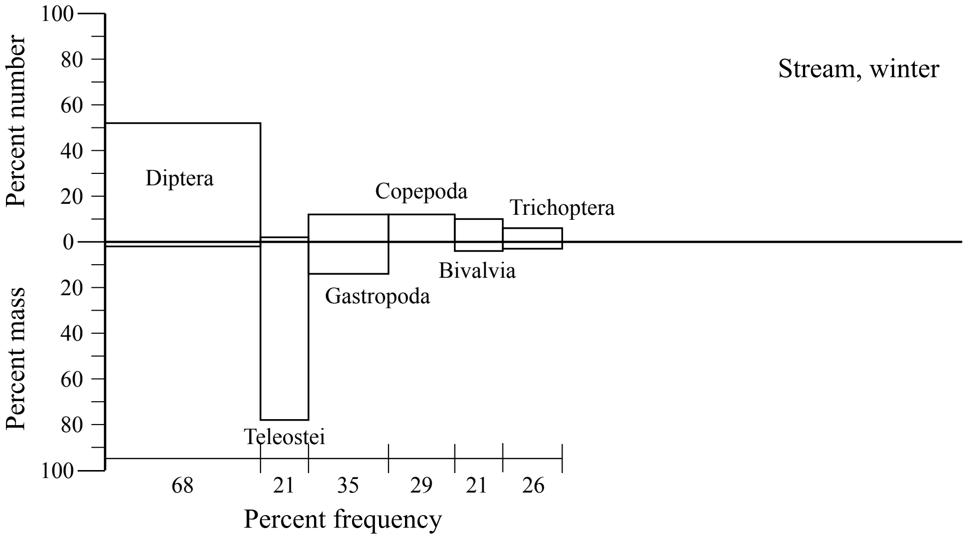

Figure 21. Prey IRI diagram for stream site, winter. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 15. Prey values for stream site, summer. Grey-colored rows are prey categories shown in IRI diagram (Fig. 19).

Stream, summer **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	643	66	4298.978	86	76	11590	89
Bivalvia	121	12	229.779	5	36	614	5
Diptera	110	11	46.368	1	38	465	4
Trichoptera	51	5	36.208	1	22	131	1
Teleostei	12	1	343.151	7	16	130	1
Cladocera	13	1	0.273	0	14	19	0
Angiospermae	6	1	6.030	0	12	9	0
Ostracoda	7	1	0.142	0	8	6	0
Coleoptera	2	0	8.823	0	4	2	0
Odonata	2	0	4.360	0	4	1	0
Insecta adult	2	0	0.513	0	2	0	0
Corixidae	1	0	3.611	0	2	0	0
Ephemeroptera	1	0	0.674	0	2	0	0
Hydracarina	1	0	0.056	0	2	0	0

Table 16. Prey values for stream site, autumn. Grey-colored rows are prey categories shown in IRI diagram (Fig. 20).

Stream, autumn **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	278	28	1655.126	76	85	8864	74
Ostracoda	505	52	7.575	0	30	1556	13
Trichoptera	96	10	146.809	7	55	909	8
Diptera	39	4	5.761	0	55	233	2
Teleostei	3	0	279.811	13	15	197	2
Corixidae	13	1	46.943	2	25	87	1
Copepoda	23	2	0.368	0	30	71	1
Bivalvia	13	1	24.687	1	25	61	1
Angiospermae	3	0	3.015	0	15	7	0
Gammaridae	3	0	1.377	0	10	4	0
Coleoptera	3	0	8.758	0	5	4	0
Collembola	1	0	0.052	0	0	0	0

Table 17. Prey values for stream site, winter. Grey-colored rows are prey categories shown in IRI diagram (Fig. 21).

Stream, winter **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Diptera	168	52	25.208	2	68	3625	51
Teleostei	8	2	1240.416	78	21	1651	23
Gastropoda	39	12	217.529	14	35	907	13
Copepoda	38	12	0.608	0	29	347	5
Bivalvia	32	10	60.768	4	21	282	4
Trichoptera	20	6	40.397	3	26	231	3
Ostracoda	10	3	0.150	0	9	27	0
Corixidae	2	1	7.222	0	6	6	0
Angiospermae	2	1	2.010	0	6	4	0
Gammaridae	2	1	0.918	0	3	2	0
Hirudinea	1	0	0.391	0	3	1	0
Hydracarina	1	0	0.056	0	3	1	0

Stomach Contents for Wetland by Season

Stomach contents of wetland blackfish varied significantly by season, including springtime (Pillai's Trace = 0.488, F (27, 276) = 1.987, p = 0.003). Ostracoda were the dominant prey group, with indices of relative importance values ranging from 34% (summer) to 77% (winter) (Fig. 22–25; Tables 18–21). More than 40% of all guts during spring and summer contained at least five different prey groups whose values were $\geq$3% IRI. Three taxa–Ostracoda, Diptera, and Gastropoda–were found in more than 80% of all guts during autumn. Copepoda were most frequently consumed during winter (40% frequency).

In the wetland during autumn, frequency of plant seed consumption was 39%; a female (wet mass 14.9 g; 101.3 mm SL), had a full stomach containing 84 plant seeds, 14 plant leaf buds, and five other prey types. Another female (wet mass 23.6 g; 115.5 mm SL) had a full stomach containing 14 plant seeds as well as eight other prey types.

Empty Guts

Wetland blackfish during wintertime had a high percentage of empty guts (esophagus + stomach + intestines), increasing from 5% in November (n = 20) to 71% in December (n = 14). By comparison with other sites, only one lake blackfish gut was empty (during summer), while one stream blackfish gut was empty (during winter). No wetland fish were harvested in January, although ten traps were soaked for several hours. (Ice thickness at that time was 76 cm, and pond water was darkly colored with a foul smell.) During February, ten traps soaked for three hours yielded only one blackfish, whose gut was empty. All ten March specimens analyzed had empty guts.

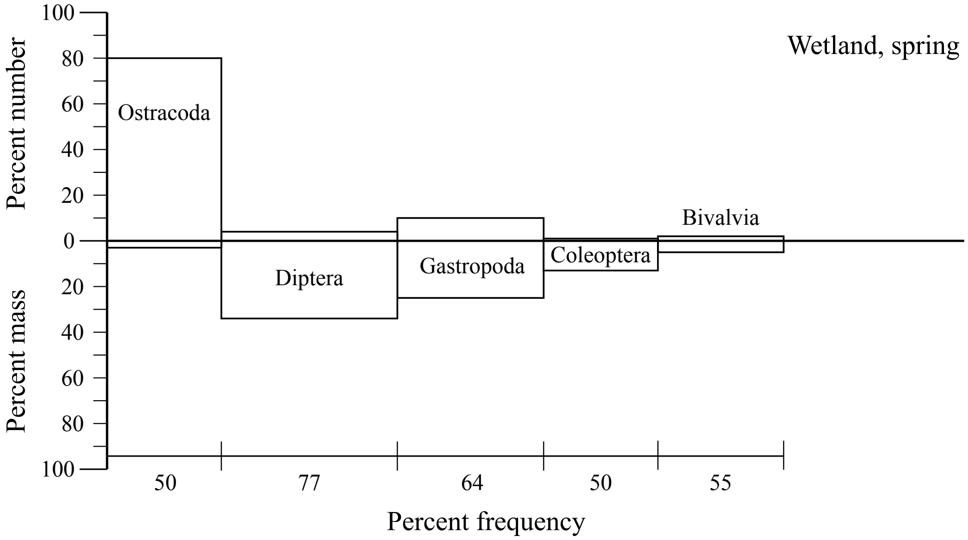

Figure 22. Prey IRI diagram for wetland site, spring. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

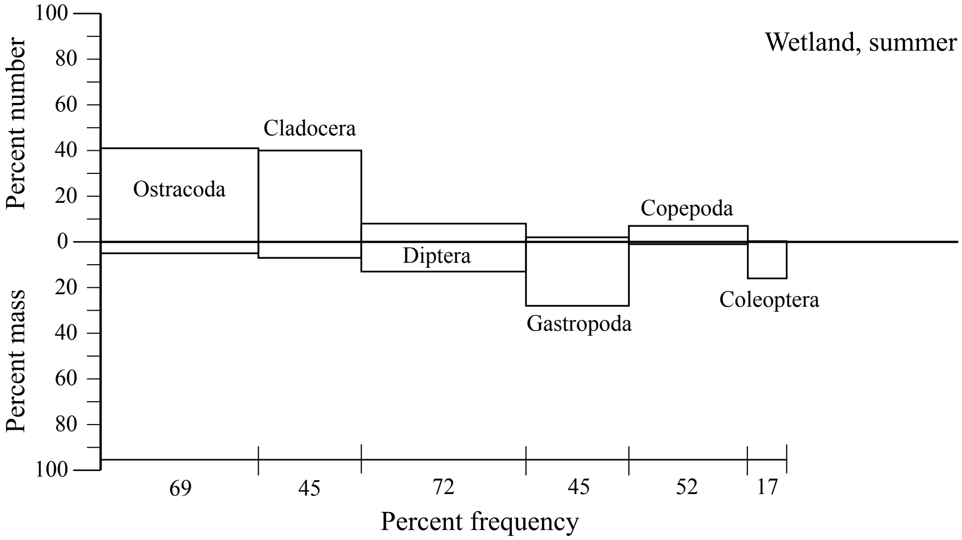

Figure 23. Prey IRI diagram for wetland site, summer. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

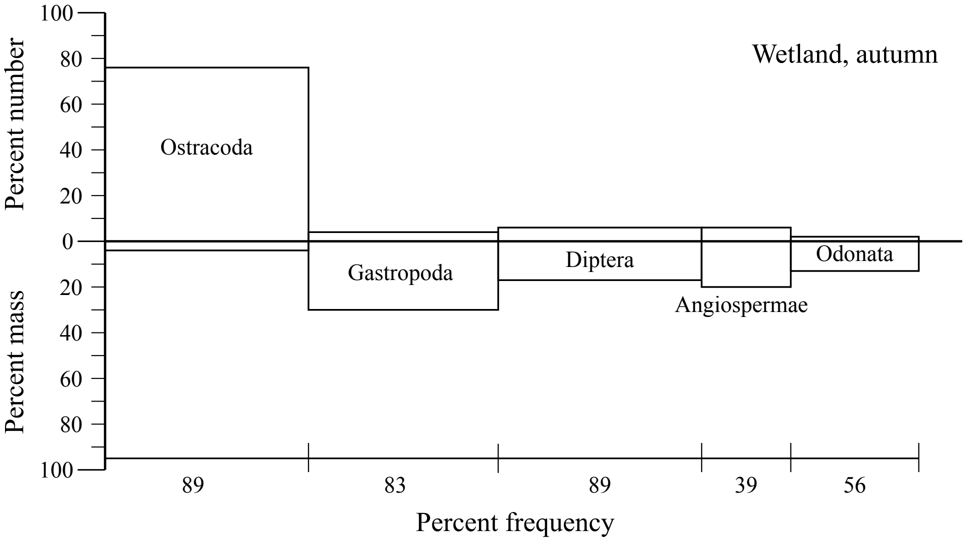

Figure 24. Prey IRI diagram for wetland site, autumn. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

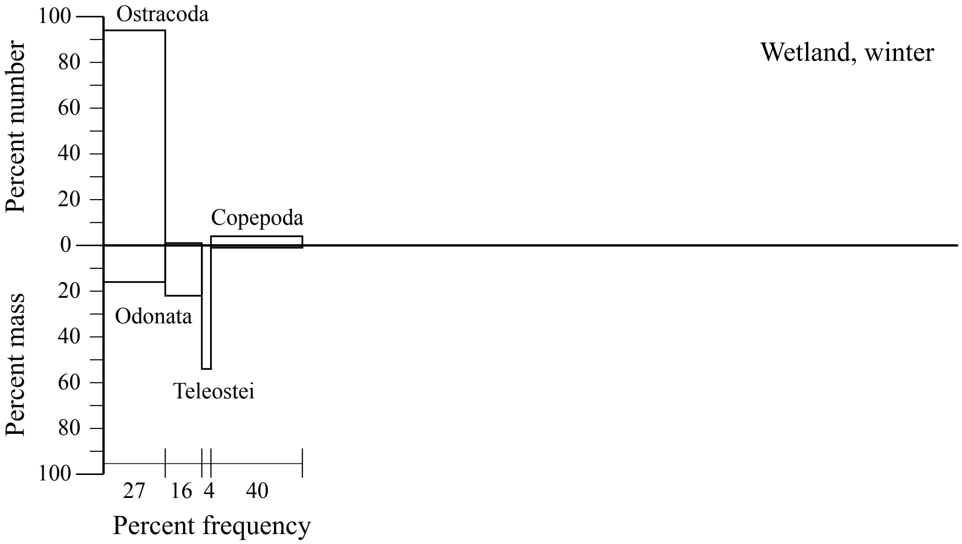

Figure 25. Prey IRI diagram for wetland site, winter. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 18. Prey values for wetland, spring. Grey-colored rows are prey categories shown in IRI diagram (Fig. 22).

Wetland, spring **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	1328	80	27.320	3	50	4153	39
Diptera	67	4	331.405	34	77	2904	27
Gastropoda	159	10	243.760	25	64	2181	20
Coleoptera	18	1	128.576	13	50	705	7
Bivalvia	28	2	53.172	5	55	386	4
Teleostei	2	0	126.205	13	9	117	1
Trichoptera	12	1	18.994	2	23	60	1
Corixidae	10	1	36.110	4	14	58	1
Angiospermae	9	1	9.045	1	23	33	0
Odonata	4	0	8.720	1	18	20	0
Copepoda	9	1	0.144	0	27	15	0
Araneae	3	0	4.238	0	14	8	0
Hydracarina	4	0	0.224	0	5	1	0
Gammaridae	1	0	0.459	0	5	0	0

Table 19. Prey values for wetland, summer. Grey-colored rows are prey categories shown in IRI diagram (Fig. 23).

Wetland, summer **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	1080	41	16.20	5	69	3157	34
Cladocera	1056	40	22.18	7	45	2097	22
Diptera	209	8	41.92	13	72	1541	16
Gastropoda	62	2	88.91	28	45	1381	15
Copepoda	184	7	2.94	1	52	406	4
Coleoptera	8	0	49.96	16	17	281	3
Angiospermae	18	1	18.09	6	31	201	2
Bivalvia	15	1	28.49	9	21	200	2
Odonata	8	0	17.44	6	17	101	1
Trichoptera	10	0	3.29	1	14	20	0
Insecta adult	2	0	7.39	2	7	17	0
Corixidae	4	0	14.44	5	3	16	0
Gammaridae	1	0	0.46	0	3	1	0
Araneae	1	0	0.20	0	3	0	0
Ephemeroptera	1	0	0.18	0	3	0	0
Unknowns	1	0	0.06	0	3	0	0
Hydracarina	1	0	0.06	0	3	0	0

Table 20. Prey values for wetland, autumn. Grey-colored rows are prey categories shown in IRI diagram (Fig. 24).

Wetland, autumn **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	1510	76	23.575	4	89	7161	49
Gastropoda	73	4	167.161	30	83	2845	19
Diptera	120	6	93.957	17	89	2060	14
Angiospermae	109	6	109.545	20	39	990	7
Odonata	33	2	71.940	13	56	821	6
Copepoda	63	3	1.008	0	72	243	2
Trichoptera	18	1	17.405	3	56	227	2
Coleoptera	6	0	41.706	8	22	176	1
Bivalvia	9	0	15.192	3	39	125	1
Cladocera	27	1	0.567	0	44	65	0
Gammaridae	6	0	2.754	1	28	22	0
Araneae	2	0	3.092	1	11	7	0
Insecta adult	1	0	0.478	0	6	1	0
Unknowns	1	0	0.277	0	6	1	0
Ephemeroptera	1	0	0.182	0	6	0	0
Hydracarina	1	0	0.056	0	6	0	0

Table 21. Prey values for wetland, winter. Grey-colored rows are prey categories shown in IRI diagram (Fig. 25).

Wetland, winter **Prey categories**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Ostracoda	4439	94	66.622	16	27	2926	77
Odonata	42	1	91.560	22	16	357	9
Teleostei	3	0	225.432	54	4	242	6
Copepoda	196	4	3.136	1	40	196	5
Diptera	27	1	2.867	1	27	34	1
Gastropoda	9	0	12.906	3	9	29	1
Angiospermae	7	0	7.035	2	2	4	0
Trichoptera	2	0	2.237	1	4	3	0
Cladocera	9	0	0.189	0	9	2	0
Bivalvia	1	0	1.899	0	2	1	0
Ephemeroptera	3	0	0.266	0	4	1	0
Gammaridae	1	0	0.459	0	2	0	0
Hydracarina	1	0	0.056	0	2	0	0

Stomach Contents by Standard Length and Sex

Standard length did not have a significant relationship to gut contents for pooled sites and seasons (Pillai's Trace = 0.063, F (9, 244) = 1.821, $p = 0.065$). Sex also did not have a significant relationship to overall diet (Pillai's Trace = 0.061, F (9, 244) = 1.756, $p = 0.077$). Prey values for males and females are included here in order to show the extent of fish consumption (Fig. 26–27; Table 22–23). Dominant prey based on %IRI values for both sexes were Gastropoda and Ostracoda, while Gastropoda was also important in terms of biomass. Guts of both males and females contained Diptera and Gastropoda at frequencies of at least 44%. While I did not detect an overall difference in the diets of males and females, males tended to consume more fish than did females. Approximately 13% of males consumed fish, which ranked third in importance by % IRI, while 6% of females consumed fish valued as 1% IRI.

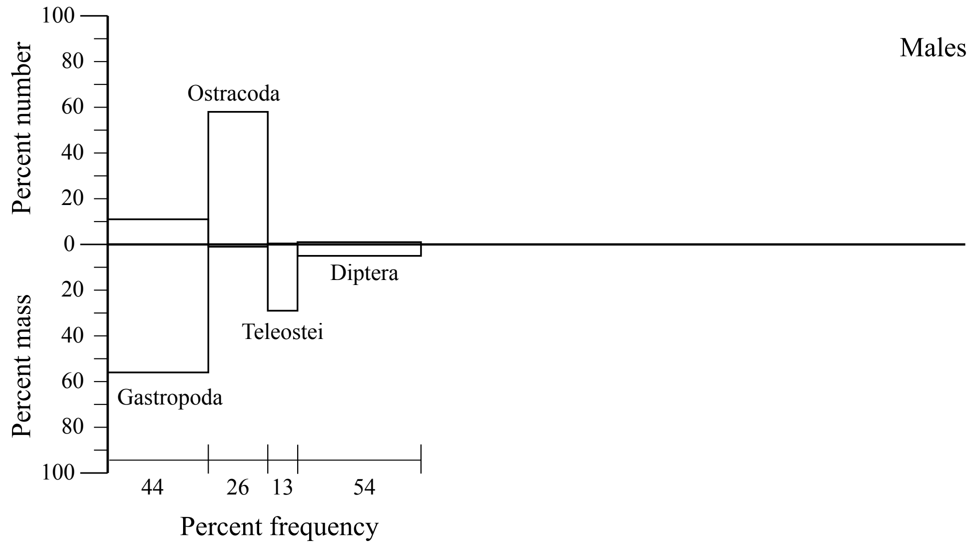

Figure 26. Prey IRI diagram for males, combined sites and seasons. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

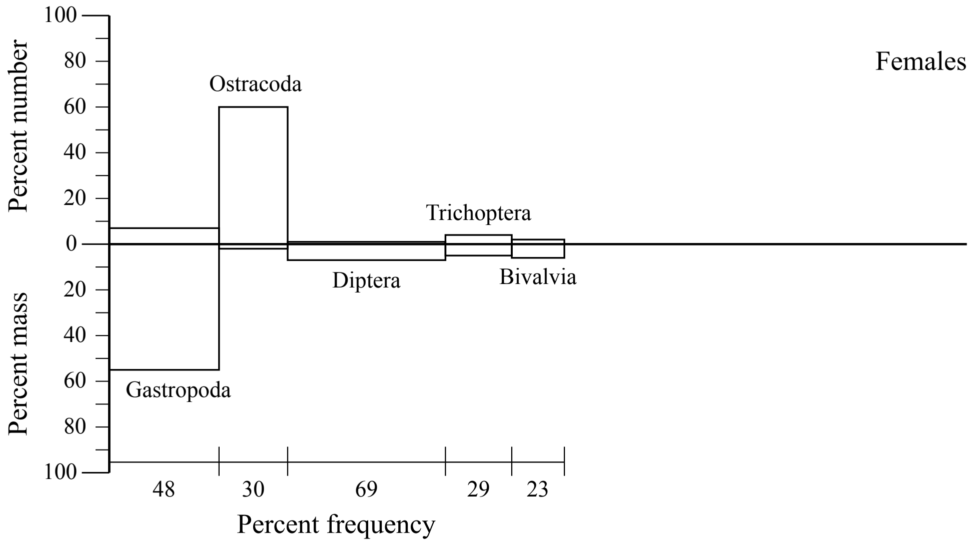

Figure 27. Prey IRI diagram for females, combined sites and seasons. Only major prey categories ≥3% IRI are shown. Frequency of Occurrence axis begins at zero for each prey category.

Table 22. Prey values for males, combined sites and seasons. Grey-colored rows are prey categories shown in IRI diagram (Fig. 26).

Males, combined sites and seasons							
Prey categories	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	687	11	4135.436	56	44	2954	52
Ostracoda	3670	58	55.124	1	26	1490	26
Teleostei	24	0	2151.309	29	13	388	7
Diptera	79	1	332.588	5	54	314	6
Cladocera	718	11	15.078	0	12	135	2
Trichoptera	190	3	141.663	2	27	132	2
Copepoda	261	4	4.176	0	28	117	2
Bivalvia	105	2	197.496	3	26	113	2
Angiospermae	37	1	37.185	1	14	16	0
Coleoptera	20	0	124.504	2	8	15	0
Odonata	28	0	61.040	1	8	10	0
Corixidae	26	0	93.886	1	6	9	0
Gammaridae	17	0	7.803	0	7	3	0
Araneae	4	0	4.038	0	3	0	0
Ephemeroptera	5	0	1.122	0	3	0	0
Plumatellida	6	0	0.540	0	2	0	0
Hydracarina	6	0	0.336	0	2	0	0
Insecta adult	2	0	3.101	0	1	0	0
Hirudinea	2	0	0.998	0	1	0	0
Collembola	1	0	0.052	0	1	0	0

Table 23. Prey values for females, combined sites and seasons. Grey-colored rows are prey categories shown in IRI diagram (Fig. 27).

Females, combined sites and seasons							
Prey categories	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	615	7	2530.951	55	48	3000	47
Ostracoda	5251	60	87.090	2	30	1858	29
Diptera	107	1	324.739	7	69	579	9
Trichoptera	321	4	210.377	5	29	242	4
Bivalvia	154	2	292.446	6	23	191	3
Copepoda	382	4	6.112	0	33	150	2
Teleostei	11	0	647.092	14	6	83	1
Cladocera	404	5	8.484	0	14	69	1
Angiospermae	135	2	135.675	3	14	65	1
Odonata	67	1	146.060	3	13	51	1
Plumatellida	423	5	38.070	1	5	30	0
Coleoptera	17	0	113.322	2	8	21	0
Corixidae	4	0	14.444	0	3	1	0
Gammaridae	10	0	4.590	0	3	1	0
Insecta adult	4	0	7.904	0	2	0	0
Hydracarina	4	0	0.224	0	3	0	0
Araneae	2	0	3.495	0	1	0	0
Unknowns	2	0	0.337	0	1	0	0
Ephemeroptera	1	0	0.182	0	1	0	0

Fish in Diet

The likelihood of fish in diet of the blackfish was related to both sex and blackfish size in the logistic regression (Chi-square = 9.487, *df* = 2, *p* = 0.009). The Wald criterion demonstrated that both sex (*p* = 0.043) and size (*p* = 0.045) made significant contributions to the model. Tests of between-subjects effects also showed one of nine variables–Teleostei–as significant by size (*F* (1, 252) = 6.678; *p* = 0.010) (Appendix B.1). Males were more likely to consume fish than females. Fish consumption first appeared in fish at least 75 mm long, although few blackfish this small had fish in their guts. Fish were more important in the diets of blackfish greater than 105 mm in length (Fig. 28; see Appendix B.2 for all prey values by size class).

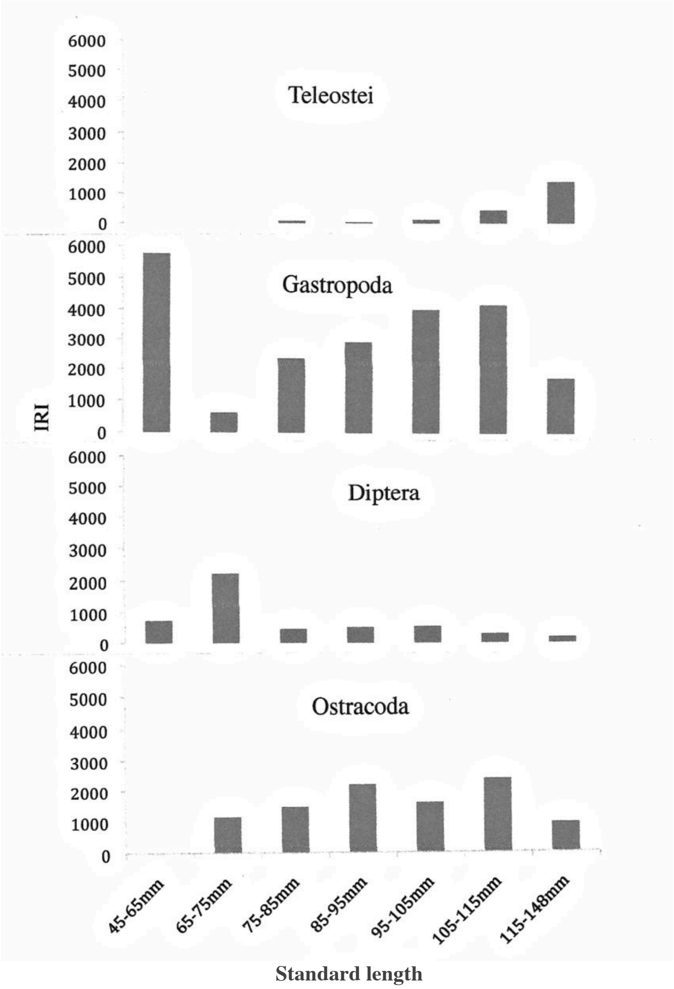

Figure 28. IRI values by standard length groupings. Four representative prey groups, including Teleostei, are shown.

Gut contents from pooled waterbodies and seasons show that nine percent of all blackfish (19 males and nine females) preyed on a total of 35 fish (Fig. 29). (See Appendix B.3 for all fish consumption data; Appendix B.4 gives a summary of all fish species other than blackfish captured in minnow traps as bycatch.) Seven percent of the lake blackfish, five males and one female, had fish in their guts–four threespine stickleback, one juvenile blackfish, and two unidentifiable fish. Rabbit Slough blackfish were the most piscivorous (17.3% frequency); 11 males and seven females consumed 10 threespine stickleback, four ninespine stickleback, one coho salmon, and three unidentified fish. The undigested juvenile coho salmon (55.3 mm TL; 1.27 mg wet mass) was in the gut of a large male blackfish (129.6 mm TL; 19.8 g wet mass) collected from the stream site in autumn. Piscivory among blackfish in the wetland was lowest at 3.5% frequency, with juvenile blackfish in the guts of two male blackfish. Overall, the frequency of cannibalism for all waterbodies and seasons was 1.3%, excluding unidentifiable prey fish. Less than one percent of blackfish had game fish in their guts.

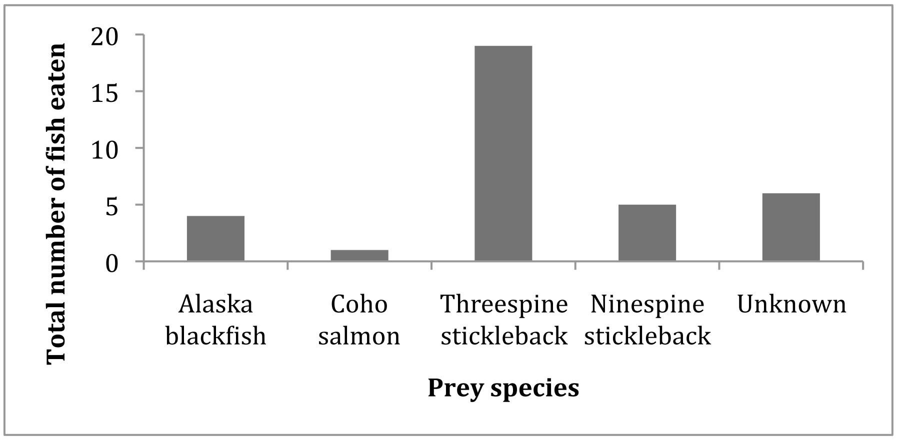

Figure 29. Total number of each prey fish species consumed by blackfish.

Discussion

The results of this food habits study indicate these introduced populations of Cook Inlet Basin blackfish are opportunistic, generalist carnivores whose diet consists primarily of benthic epiphytic invertebrates–gastropods, ostracods, and dipteran larvae. While their diets were varied, I did not detect surface feeding in these poulations, and consumption of adult insects was rare. Additionally, piscivory (including cannibalism) was infrequent in these populations.

Analysis of stomach contents indicated that site and season were significant in explaining diet variation, while standard length and sex were not significant in explaining diet variation. While lake blackfish consumed mainly dipteran larvae, stream blackfish consumed mainly gastropods, and wetland blackfish consumed mostly ostracods. Important prey based on biomass varied, from teleosts among lake fish to gastropods among stream and wetland fish. Such spatial variations in the food habits of blackfish support the trophic model of a generalist feeder, whose diet consists mostly of benthic invertebrates with diverse sizes and structures, selected based in part on availability. Trophic shifts to fish as prey indicate opportunism to maximize energy intake, a strategy common to many fishes (Gerking 1994).

Diets also varied significantly across time, as expected. Gastropods were the most important prey based on IRI, mass, and frequency of consumption during summer. Diet shifted to gastropods and ostracods during autumn, with dipteran larvae most frequently consumed. Winter prey consisted of ostracods, dipteran larvae, and teleosts. These temporal prey shifts support a trophic model of adaptability based on seasonal prey availability, enabling blackfish to successfully colonize multiple freshwater habitats, with significant potential ecological impacts.

Predicted temporal diet shifts also occurred within each waterbody. Lake fish selected teleosts, dipteran larvae, and trichopteran larvae during summer but shifted to primarily trichopteran larvae during autumn, while stream fish consumed mainly gastropods during summer and autumn. The wetland site was the only site studied to include springtime prey values. Ostracods, dipteran larvae, and gastropods were the most important prey categories during springtime in the wetland, with diet shifting to ostracods during summer and autumn. Both lake fish and stream fish continued feeding during winter but at reduced intensities, based on smaller IRI values during winter. Dipteran larvae were the most important winter prey based on IRI values, for both lake fish and stream fish. By foraging during winter in the lake and stream sites, introduced blackfish in the Cook Inlet Basin exploit a broad niche (Chilton *et al*. 1984) and therefore may adversely impact native prey species, which might otherwise reach larger population sizes if predation pressure ceased during periods of ice and snow cover.

Unlike lake and stream foragers, most wetland blackfish stopped feeding in winter, based on the high percentage of empty guts dissected. Trapping times yielded few to no fish at the wetland site during some winter months. Although excluded from this study, morphologies of dissected esophaguses from wintertime wetlands blackfish were noted. Esophageal tissues of some wintertime wetland blackfish with empty guts were distinctly thin and translucent with large, prominent veins, in contrast to all lake and stream blackfish as well as wetland blackfish collected during other seasons, whose esophaguses consisted of thicker, opaque tissues and smaller veins. Additional physiological and ecological investigations are needed to explain these morphologies, which may relate to survival mechanisms in freezing, hypoxic waters, especially oxygen uptake. Blackfish burrow into sediment to escape predation (personal observation), but such winter survival mechanisms are anecdotal.

Some blackfish opportunistically consumed plant seeds that perhaps resembled small invertebrates such as seed shrimp (ostracods). Examination of intestinal contents showed no apparent digestion of the hard outer seed coats. Occasional plant pieces including stems and leaves were considered accidental ingestions; however, one noteworthy exception was a wetland blackfish during autumn that ate 14 plant buds in addition to 84 plant seeds, 40 ostracods and a few minor invertebrates. The short gastrointestinal tract of the blackfish adequately processes animal prey but is too short for digestion of plant cellulose (Barton 2007).

These results support the model of the blackfish as a generalist opportunist that feeds on a wide size range and morphology of prey species from more than one trophic level. Such low dietary specialization is characteristic of most successful fish invaders (Moyle and Light 1996a, 1996b, Marchetti *et al*. 2004, Gido and Franssen 2007).

Fish Consumption

Fish consumption was infrequent among these populations, though still important in blackfish diet in terms of total prey mass. A single salmonid, a juvenile coho, was eaten, and given its undigested state, it may have been consumed while inside the trap. Seven total prey fish (two blackfish, three ninespine stickleback, and one threespine stickleback) were undigested, also indicating possible predation while inside the trap (Moyle 1977), compared to five fish which were mostly or completely digested, indicating they were eaten prior to trapping. Interestingly, threespine stickleback eggs were absent from blackfish stomach contents, although stickleback spawn in benthic nests in lentic waters. Blackfish in these three introduced populations do not appear to present a major direct predation threat to game fish populations in the Cook Inlet Basin.

In contrast, other studies reported dominance of fishes in blackfish diet. Stomachs of adult blackfish from western Alaska contained mostly small blackfish and northern pike (Baxter 1973, unpublished, cited in Chlupach 1975). Stomach contents of 320 blackfish electrofished from Meadow Lake (Anchorage) contained 132 fish identified as Salmoniformes (Chlupach 1975).

Comparative Diet Studies

In another study, gut contents of 77 blackfish collected during summer at Point Barrow on the Arctic Coastal Plain contained 17 prey categories including nematodes and algae (Ostdiek and Nardone 1959). In the current study, 106 Cook Inlet Basin blackfish guts from summer contained 16 prey categories, excluding nematodes and algae. The most frequently consumed prey of Point Barrow blackfish were cladocerans (91%), dipteran larvae (90%), and ostracods (88%); fish consumption (species unlisted, though stickleback were reported for the site) occurred at less than 3% frequency.

By comparison, Cook Inlet Basin blackfish during summer most frequently ate gastropods (57%) and dipteran larvae (54%), while fish consumption occurred at a frequency of 11%. Diet variation between the two regions may be partly due to prey availability at the specific sites as well as blackfish size. Point Barrow blackfish were smaller (71.7 mm mean TL) than Cook Inlet Basin blackfish collected during summer (112 mm mean TL).

A study of 320 Meadow Lakes (Anchorage) blackfish harvested during September reported gut contents as follows: major prey by relative frequency, Cladocera (59%) and Copepoda (32%), and six minor prey valued at less than 5% frequency (Hemiptera, Diptera, Odonata, Teleostei, Mollusca, and Ephemeroptera). By comparison, 67 Cook Inlet blackfish collected in autumn ate Diptera (79%), Gastropoda (69%), and Trichoptera (63%) as well as six other prey types valued between 10–40%. These results support the broad model of the blackfish as a trophic generalist feeder whose prey consists mainly of benthic invertebrates but may include fish when available.

Management Implications

Dietary Overlap

Diets of introduced blackfish in Cook Inlet Basin freshwaters overlap with those of native fishes and stocked sportfish. Threespine stickleback feed on small benthic invertebrates including dipteran larvae, ostracods, molluscs, copepods, cladocerans, and amphipods (Hynes 1950; Greenbank and Nelson 1959), while slimy sculpins select slightly larger organisms on or just below the sediment–amphipods and larvae of dipterans, trichopterans, and odonates (Morrow 1980, Flecker 1984, Hershey 1985). Stickleback and sculpins are the two native species with feeding behaviors very similar to those of blackfish.

Juvenile Dolly Varden char forage on small crustaceans, insect larvae, snails, clams, spiders, and fish (Morrow 1980). Coho salmon fry consume microzooplankton, mites, collembola, and spiders, while larger juveniles also eat adult beetles (Morrow 1980). Similarly, blackfish consume diverse epiphytic benthic prey with a wide range of structures and sizes. In contrast to blackfish, coho salmon fry feed heavily on surface insects including winged dipterans and trichopterans, and large adults can also become primarily piscivorous (Morrow 1980). Blackfish swim to the surface to breathe atmospheric air but are not known to eat surface insects.

Rainbow trout feeding habits also overlap with those of blackfish, with some exceptions. Rainbow trout shift ontogenetically from cladocerans for small juveniles to dipteran larvae and winged adults, leeches, amphipods, gastropods, water beetles, and fishes for large adults (Scott and Crossman 1973, Morrow 1980, Beauchamp 1990). Rainbow trout feed at the surface, in mid current, and sometimes at the bottom. Blackfish feed demersally by picking organisms off of benthic macrophytes or by probing sediment in search of buried clams and large dipteran larvae, using their protruding lower jaw like a scoop (personal observation). In contrast, rainbow trout do not burrow for prey (Frost and Brown 1967, Knapp *et al*. 2001).

Blackfish as an Invasive Species

Fish introductions can cause dramatic changes in benthic macroinvertebrate communities (Gerking 1994, Knapp *et al*. 2001). Studies have found that introduced trout significantly decreased overall benthic biomass including larvae of dipterans and trichopterans (Macan 1966, 1977). Brook trout (*Salvelinus fontinalis*) stocked in a fishless lake in New York immediately impacted the benthic fauna, including eliminating *Chaborus* dipteran larvae (Gloss *et al*. 1989). Selective feeding by introduced yellow perch (*Perca flavescens*) in a lake in Quebec, Canada, changed the overall community structure, resulting in reduced populations of larger invertebrates which produced more abundant populations of smaller invertebrates (Post and Cucin 1984; Berglund 1968; Crowder and Cooper 1982). As expected, removal of a fish predator in some cases caused measurable increases in benthic organisms (Gerking 1994).

Benthivorous blackfish are documented in large numbers in some Cook Inlet Basin waterbodies (K. Dunker, ADF&G, personal communication; personal observation). Within DeLong Lake, they are found among dense stands of rooted *Elodea* (personal observation), a highly invasive macrophyte that provides ideal cover, prey habitat, and possibly also spawning habitat for blackfish (Aspenwall 1965). While direct predation of salmonids by blackfish was extremely rare in this study, the substantial diet overlap with native and game fishes along with known impacts of other introduced fishes on benthic invertebrate communities support the recognition of Cook Inlet Basin blackfish as an invasive species likely to cause significant ecological impacts.

Management Recommendations

"The participation of the public in environmental decision-making and management is…essential for the success of conservation initiatives." (Fischer and Young 2007). While trapping blackfish for this study, I frequently encountered people who had never heard of blackfish, while others thought they were burbot (*Lota lota*; family Lotidae). A comprehensive survey of area lakes, streams, ponds, and wetlands for blackfish presence could help managers assess the rate of colonization over the past 60 years. An extensive public awareness campaign could include informational signs posted at popular angling lakes and streams featuring a species description and invasive species status, as well as a warning to kill any blackfish caught. A social media site could be available for anglers to report blackfish bycatch, and public outreach presentations at local schools could help educate and inform citizens of the risks of ecosystem alterations by blackfish. Finally, funding for continued research on blackfish ecology, reproduction, developmental anatomy, and physiology would add to our body of knowledge about this poorly studied species, so that complex trophic relationships with blackfish in freshwater ecosystems of the Cook Inlet Basin might be better understood.

Conclusion

Fish diet analysis can help to define trophic interactions within aquatic food webs, thus serving as a powerful tool to evaluate the effects of the establishment of an introduced species. The aims of this study were to describe the feeding ecology of introduced populations of Cook Inlet Basin blackfish by analyzing diet composition across space and time as well as sex and body size. Blackfish are well-established invaders of Cook Inlet Basin fresh waters, and results presented here show they are generalist benthivores whose major prey consists of dipteran larvae, gastropods, and ostracods. Minor prey consists of 17 different prey taxa, some of which are consumed only rarely, and fish consumption, including cannibalism, is infrequent in these populations. Blackfish diet overlaps extensively with the diets of native and sport fishes. Blackfish are active and consuming prey year-round in some waterbodies. This suggests that blackfish may impact native and sport fishes through resource competition, as well as impacting their broad prey base and hence community structure through predation. Fisheries managers should attempt to restrict the further spread of blackfish through public awareness campaigns, law enforcement, and the facilitation of research on control measures.

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Appendix A

Abbreviations Used

C	Celsius
Cl.	class
cm	centimeter
D	dark
Div.	division
est.	estimated
F	Fahrenheit, family, female
g	gram
gal	gallon
hr	hour
in	inch
Infrcl.	infraclass
L	liter, light
m	meter
M	male
mg	milligram
mL	milliliter
mm	millimeter
n, N	number
ns	statistically not significant
Or.	order
Ph.	phylum
ppm	parts per million
ppt	parts per thousand
RO	reverse osmosis
SD	standard deviation
SE	standard error
SL	standard length (end of snout to end of last vertebra, excluding caudal fin)
Subor.	suborder
Subph.	subphylum
T	tablespoon
TL	total length (end of snout to end of tail)
tsp	teaspoon
YOY	young of the year

Appendix B

Diet Analysis Supplemental Tables

Table B.1. Tests of between-subjects effects. Model consists of nine dependent variables – masses (M) of prey greater than or equal to 1%IRI; independent variables are site, season, and sex; and covariates are standard length (SL) and trapping hours.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
SL	GastropodaM	3211.785	1	3211.785	.824	.365
	OstracodaM	1.074	1	1.074	.244	.622
	DipteraM	8.270	1	8.270	1.148	.285
	TeleosteiM	11203.744	1	11203.744	6.678	.010
	TrichopteraM	21.717	1	21.717	1.026	.312
	BivalviaM	80.595	1	80.595	2.491	.116
	CopepodaM	.006	1	.006	.462	.497
	CladoceraM	.967	1	.967	2.266	.133
	AngiospermM	15.512	1	15.512	.627	.429
Trap Hrs.	GastropodaM	33112.410	1	33112.410	8.496	.004
	OstracodaM	2.312	1	2.312	.525	.469
	DipteraM	.001	1	.001	.000	.989
	TeleosteiM	230.902	1	230.902	.138	.711
	TrichopteraM	60.533	1	60.533	2.859	.092
	BivalviaM	102.895	1	102.895	3.180	.076
	CopepodaM	.001	1	.001	.078	.780
	CladoceraM	.234	1	.234	.548	.460
	AngiospermM	.161	1	.161	.006	.936
Site	GastropodaM	137207.149	2	68603.574	17.603	.000
	OstracodaM	17.321	2	8.661	1.966	.142
	DipteraM	49.745	2	24.873	3.451	.033
	TeleosteiM	5340.744	2	2670.372	1.592	.206
	TrichopteraM	250.855	2	125.428	5.923	.003
	BivalviaM	205.903	2	102.952	3.181	.043
	CopepodaM	.066	2	.033	2.408	.092
	CladoceraM	3.428	2	1.714	4.016	.019
	AngiospermM	180.355	2	90.178	3.648	.027

Table B.1. …Continued

Season	GastropodaM	30091.526	2	15045.763	3.861	.022
	OstracodaM	5.192	2	2.596	.589	.556
	DipteraM	47.152	2	23.576	3.271	.040
	TeleosteiM	8992.393	2	4496.197	2.680	.071
	TrichopteraM	619.829	2	309.914	14.636	.000
	BivalviaM	190.140	2	95.070	2.938	.055
	CopepodaM	.022	2	.011	.797	.452
	CladoceraM	.773	2	.386	.905	.406
	AngiospermM	178.311	2	89.155	3.607	.029
Sex	GastropodaM	124.575	1	124.575	.032	.858
	OstracodaM	15.427	1	15.427	3.501	.062
	DipteraM	4.078	1	4.078	.566	.453
	TeleosteiM	5171.900	1	5171.900	3.083	.080
	TrichopteraM	91.689	1	91.689	4.330	.038
	BivalviaM	3.862	1	3.862	.119	.730
	CopepodaM	.017	1	.017	1.272	.260
	CladoceraM	.167	1	.167	.392	.532
	AngiospermM	92.693	1	92.693	3.750	.054
Waterbody * Season	GastropodaM	68647.372	4	17161.843	4.404	.002
	OstracodaM	9.688	4	2.422	.550	.699
	DipteraM	261.597	4	65.399	9.075	.000
	TeleosteiM	8043.710	4	2010.928	1.199	.312
	TrichopteraM	350.182	4	87.546	4.134	.003
	BivalviaM	203.710	4	50.928	1.574	.182
	CopepodaM	.060	4	.015	1.097	.358
	CladoceraM	6.316	4	1.579	3.700	.006
	AngiospermM	379.711	4	94.928	3.840	.005

Table B.2. Prey values for size classes, combined sites and seasons.

Size class I, 45-65mm SL Prey category	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Gastropoda	29	63	122.916	86	50	5764	83
Diptera	6	13	8.923	6	60	738	11
Trichoptera	5	11	3.47	2	30	224	3
Bivalvia	4	9	7.596	5	20	187	3
Cladocera	2	4	0.042	0	20	41	1

Size class II, 65-75mm SL Prey category	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Diptera	16	4	16.006	28	70	2227	45
Ostracoda	147	39	2.242	4	26	1127	23
Gastropoda	13	3	14.158	25	22	609	12
Trichoptera	29	8	6.615	11	22	418	8
Odonata	5	1	10.900	19	13	264	5
Copepoda	64	17	1.024	2	13	246	5
Bivalvia	2	1	3.798	7	4	31	1
Cladocera	5	1	0.105	0	17	26	1
Insecta adult	1	0	2.623	5	4	21	0
Ephemeroptera	1	0	0.182	0	4	3	0
Hydracarina	1	0	0.056	0	4	2	0

Size class III, 75-85mm SL Prey category	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Gastropoda	131	6	540.517	53	40	2362	45
Ostracoda	1174	56	17.647	2	25	1460	28
Diptera	34	2	59.742	6	62	461	9
Trichoptera	219	10	74.592	7	25	450	9
Bivalvia	52	2	98.748	10	20	242	5
Copepoda	100	5	1.6	0	40	196	4
Teleostei	2	0	193.032	19	4	69	1
Angiospermae	9	0	9.045	1	15	19	0
Cladocera	12	1	0.252	0	15	9	0
Odonata	5	0	10.9	1	5	7	0
Coleoptera	2	0	15.14	1	4	6	0
Plumatellida	14	1	1.26	0	7	6	0
Insecta adult	2	0	0.513	0	2	0	0

Table B.2. …Continued

Size Class IV, 85-95mm SL **Prey category**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	225	7	1304.222	67	40	2920	48%
Ostracoda	2582	77	46.463	2	28	2191	36%
Diptera	35	1	146.350	8	60	516	8%
Bivalvia	59	2	112.041	6	22	168	3%
Copepoda	84	3	1.344	0	36	93	2%
Odonata	40	1	87.200	4	14	78	1%
Trichoptera	45	1	34.544	2	24	75	1%
Teleostei	3	0	126.992	7	5	34	1%
Cladocera	68	2	1.428	0	12	25	0%
Coleoptera	7	0	45.434	2	7	18	0%
Corixidae	8	0	28.888	1	5	9	0%
Angiospermae	10	0	10.050	1	10	8	0%
Gammaridae	3	0	1.377	0	5	1	0%
Hydracarina	2	0	0.112	0	3	0	0%
Araneae	1	0	1.922	0	2	0	0%
Insecta adult	1	0	0.478	0	2	0	0%
Unknowns	1	0	0.060	0	2	0	0%
Collembola	1	0	0.052	0	2	0	0%
Ephemeroptera	1	0	0.040	0	2	0	0%

Size Class V, 95-105mm SL **Prey category**	**Total no.**	**% no.**	**Total mass (mg)**	**% mass**	**% freq.**	**IRI**	**% IRI**
Gastropoda	372	10	2171.644	63	54	4017	58%
Ostracoda	2002	56	30.03	1	28	1591	23%
Diptera	43	1	253.52	7	63	544	8%
Trichoptera	101	3	51.48	2	32	140	2%
Copepoda	171	5	2.736	0	28	136	2%
Angiospermae	111	3	111.555	3	21	131	2%
Cladocera	343	10	7.203	0	13	130	2%
Teleostei	5	0	545.116	16	7	118	2%
Bivalvia	54	2	102.546	3	24	106	2%
Odonata	22	1	47.96	1	12	24	0%
Coleoptera	12	0	73.046	2	9	22	0%
Gammaridae	9	0	4.131	0	7	3	0%
Corixidae	5	0	18.055	1	3	2	0%
Insecta adult	1	0	4.768	0	1	0	0%
Plumatellida	2	0	0.18	0	1	0	0%
Hirudinea	1	0	0.607	0	1	0	0%
Ephemeroptera	1	0	0.182	0	1	0	0%
Hydracarina	1	0	0.056	0	1	0	0%

Table B.2. …Continued

Size Class VI, 105-115mm SL Prey category	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Gastropoda	371	11	1923.729	58	60	4175	52%
Ostracoda	2052	59	30.817	1	40	2380	30%
Teleostei	13	0	803.965	24	17	429	5%
Diptera	33	1	125.088	4	62	296	4%
Bivalvia	46	1	85.455	3	43	170	2%
Trichoptera	54	2	108.726	3	30	147	2%
Copepoda	139	4	2.224	0	34	138	2%
Cladocera	231	7	4.851	0	13	90	1%
Coleoptera	13	0	87.813	3	17	52	1%
Plumatellida	246	7	22.14	1	4	29	0%
Angiospermae	22	1	22.11	1	19	25	0%
Odonata	11	0	23.98	1	13	14	0%
Corixidae	10	0	36.11	1	6	8	0%
Gammaridae	14	0	6.426	0	11	7	0%
Araneae	5	0	5.611	0	9	3	0%
Ephemeroptera	3	0	0.9	0	4	0	0%
Hydracarina	2	0	0.112	0	4	0	0%
Hirudinea	1	0	0.391	0	2	0	0%
Unknowns	1	0	0.277	0	2	0	0%

Size Class VII, 115-148mm SL Prey category	Total no.	% no.	Total mass (mg)	% mass	% freq.	IRI	% IRI
Gastropoda	166	8	620.631	31	46	1763	35%
Teleostei	12	1	1060.226	52	26	1395	27%
Ostracoda	964	46	15.015	1	20	929	18%
Trichoptera	59	3	74.643	4	31	204	4%
Cladocera	462	22	9.702	0	9	192	4%
Diptera	20	1	47.988	2	57	190	4%
Bivalvia	45	2	85.455	4	29	182	4%
Copepoda	85	4	1.36	0	26	105	2%
Plumatellida	167	8	15.03	1	11	99	2%
Angiospermae	20	1	20.1	1	14	28	1%
Corixidae	7	0	25.277	1	11	18	0%
Odonata larvae	12	1	26.16	1	9	16	0%
Coleoptera	3	0	16.393	1	6	5	0%
Gammaridae	2	0	0.918	0	6	1	0%
Hydracarina	4	0	0.224	0	3	1	0%
Insecta adult	1	0	2.623	0	3	1	0%

Table B.3. Prey fish consumption with corresponding predator data. See abbreviation key at bottom of table. Rows with an asterisk (*) denote same blackfish predator.

Site	Season	Prey Species	Prey lifestage	Count	Degree of Digestion	Trapping time, hrs.	Size of Predator (mm SL)	Sex of Predator
Lake	Summer	THRSPN	U	1	C	13.5	91.3	M
Lake	Summer	UNK	J	1	P	13.5	85.0	F
Lake	Summer	UNK	J	1	P	13.5	100.2	M
Lake	Summer	THRSPN	U	1	U	13.5	96.6	M
Lake	Autumn	THRSPN	U	1	C	3.0	123.7	M
Lake*	Winter	BLK	J	1	U	4.0	123.8	M
Lake*	Winter	THRSPN	U	1	P	4.0	123.8	M
Stream	Summer	THRSPN	S	2	P	13.5	119.1	M
Stream	Summer	UNK	J	1	P	13.5	105.7	M
Stream	Summer	THRSPN	J	2	P	13.5	106.8	F
Stream	Summer	THRSPN	J	1	P	13.5	84.7	F
Stream	Summer	THRSPN	J	1	P	13.5	78.4	F
Stream	Summer	THRSPN	J	1	P	6.0	114.6	M
Stream	Summer	THRSPN	J	2	P	6.0	108.3	F
Stream	Summer	THRSPN	J	2	M	6.0	109.9	M
Stream	Autumn	UNK	U	1	P	9.0	115.9	M
Stream	Autumn	COHO	J	1	U	3.5	109.5	M
Stream	Autumn	UNK	J	1	P	3.5	115.5	M
Stream	Winter	NINSPN	A	2	P	3.5	115.5	M
Stream	Winter	NINSPN	A	1	U	3.5	117.5	M
Stream	Winter	THRSPN	J	1	P	3.5	121.2	F
Stream	Winter	THRSPN	A	1	P	3.5	97.9	M
Stream	Winter	NINSPN	S	1	U	3.5	107.6	F
Stream	Winter	NINSPN	A	1	U	3.5	109.8	M
Stream	Winter	BLK	J	1	U	3.5	96.6	F
Wetlands	Spring	BLK	J	1	P	5.0	123.1	M
Wetlands	Spring	UNK	U	1	M	5.5	94.4	F
Wetlands	Winter	THRSPN	A	2	M	3.5	107.3	M
Wetlands	Winter	BLK	J	1	P	3.0	100.9	M

PREY SPECIES KEY:	PREY LIFESTAGE KEY:	DEGREE OF DIGESTION KEY:
BLK-blackfish	U-unknown	U-undigested
COHO-Coho salmon	J-juvenile	P-partially digested
NINSPN-ninespine stickleback	S-subadult	M-mostly digested
THRSPN-threespine stickleback	A-adult	C-completely digested (bony parts only)
UNK-unknown species		

Table B.4. Fish bycatch recorded for all trappings. If known, exact counts are given. P = present in trap, but count was not recorded. YOY = young-of-the-year life stage.

SITE	MONTH	Threespine Stickleback	Juvenile Coho Salmon	Ninespine Stickleback	Other Species	Notes
Lake	SEP	> 25				(All fish inside one trap placed just below surface.)
Lake	DEC	1			1*	*Rainbow trout, est. TL = 15 cm.
Stream	JAN		12			
Stream	FEB		1			
Stream	MAR		3			
Stream	MAY	9	1	9		
Stream	JUL	P	P			
Stream	JUL	P*				(*Abundant YOY)
Stream	JUL	P*	P			(*YOY, one gravid female)
Stream	AUG	P*	45**			(*YOY) (**Sizes vary)
Stream	AUG	P*			1**	(*YOY, adults) (**Slimy sculpin)
Stream	SEP		6			
Stream	OCT	P	P*			(*Abundant, sizes vary)
Stream	NOV		1			
Stream	NOV	5	2			
Stream	DEC		3			
Stream	JUL	P*	14	1		(*YOY, one adult female)
Wetland	MAY			1		
Wetland	NOV	1				

Appendix C

Alaska Blackfish Husbandry Manual

Overview

The Alaska blackfish (*Dallia pectoralis;* family Esocidae; hereafter blackfish*)*, is a small freshwater teleost found only in Alaska and Siberia. Extremely hardy due to its ability to breathe atmospheric air and survive freezing water, the colorfully patterned blackfish makes an ideal aquatic vertebrate to keep in both classroom and laboratory aquaria, for investigations ranging from behavior and development to physiology and toxicology. This species is poorly understood for its survival mechanisms in Arctic waters, and holding live blackfish for research enables scientists to better understand their unique adaptations. The lifespan of blackfish, though not yet documented, is at least 5–7 years based on specimens kept for this study at the University of Alaska Anchorage (UAA). Blackfish can be repeatedly handled out of water for short periods of time without harm to the fish, and can survive for years in aquaria if properly cared for.

Live blackfish in the classroom make ideal freshwater specimens for teaching young students about fish biology and behavior. Blackfish can serve as models for educating children about invasive species and the harmful effects of releasing aquarium fish into the wild. Students can experience holding hardy blackfish out of water for short periods of time without harm to the fish, a unique opportunity they do not get with fish that cannot breathe atmospheric air. From personal observation, holding a live blackfish is a big hit with both children and adults. Blackfish are sturdy enough to keep for years in classroom aquaria, and young students can be assigned aquarium maintenance tasks to learn how to responsibly care for live animals. Anyone who collects and keeps live blackfish must be well informed regarding state laws and permit requirements, follow methods for ethical handling of live aquatic vertebrates, and most importantly practice good stewardship by never releasing live animals back into the wild.

As a graduate student in Dr. Frank von Hippel's ecology lab at UAA, I kept live blackfish in lab aquaria from 2009–2012. Blackfish were collected and held in plastic wading pools and 757 L (200 gal) circular tanks outdoors during summer, as well as 303 L (80 gal) fiberglass tanks indoors year-round. Smaller glass tanks were also used for juvenile blackfish. Temperature, photoperiod, and salinity were manipulated to determine best conditions for keeping blackfish in the lab. A variety of tank substrates, including bare-tank, sand, gravel, washed sphagnum moss, and live moss, were tried in order to determine those that best duplicated the natural environment and were simultaneously easy to clean. Feeds I tried included dried flake, freeze-dried plankton, frozen fish, frozen adult brine shrimp, frozen bloodworms, and live invertebrates. One small cohort was produced through *in vitro* fertilization.

The following manual is written for researchers and educators wishing to keep live blackfish in aquaria for extended periods. Here I describe tank set-up and maintenance, water quality, photoperiod and temperature, stocking density, feeding, and handling mortalities. Captive fish behavior is discussed, and artificial fertilization of wild-caught blackfish is detailed.

Supply List

Glass or fiberglass tanks
Tank covers
Full-spectrum fluorescent lights
Synthetic sea salt
Sponge filters
Airline tubing
Air pump
Air valves
Dried moss
10 cm (4 in) PVC elbows
Thermometer

Water test kit
Chiller
Submersible pump
Aquarium nets
Feed: Frozen bloodworms, live bloodworms, live snails, live glass shrimp
Disposable gloves
Plastic 19 L (5 gal) buckets

Supply List for Artificial Fertilization and Larval Rearing

Petri dishes
Dissecting tools
Disposable gloves
MS-222 fish anesthetic buffered with baking soda
Methylene blue
One-liter glass jars
Air stones
Air pump
Brine shrimp cysts
Synthetic sea salt
Submersible aquarium heater
38 L (10 gal) glass aquaria

Tank Set-up

I use custom-made 303 L (80 gal) fiberglass tanks with plexiglass viewing windows. Prior to use, all tanks should be checked for leaks. Fill each tank with water, mark with a permanent marker any leak locations on the outside of the tank, drain, dry, and apply silicone cement to the exterior. Let cement dry. Fill tank with cold tap water. Add synthetic sea salt dissolved in tap water to adjust salinity to 0.5–1.0 ppt. There is no need to adjust pH. Use four large sponge filters per 303 L (80 gal) tank. Attach airline tubing and connect to air source. Adjust air valve to create a moderate stream of bubbles, and aerate tank for several days to remove chlorine before introducing fish. Avoid strong currents in blackfish tanks. Tanks must be covered—blackfish jump! I use plastic "egg crate" light covers available from home improvement stores. Covers are easily cut with serrated office scissors to 61 cm (24 in) widths, which allows for sections to be easily removed to feed and maintain fish. Blackfish require abundant places to hide, to reduce stress. Refuges can consist of tunnels made with black 10 cm (4 in) PVC elbows. (Glue on rocks with aquarium cement if PVC elbows float.) Rock tunnels can be made by gluing pieces of slate together with aquarium cement. Because blackfish naturally burrow into benthic silt and among plants in their natural habitat, I prefer to use something to simulate natural submerged vegetation. Sphagnum moss makes an ideal substrate for captive-held blackfish (J. Wetzel, Lincoln University, personal communication), and provides ample refuge to reduce fish stress. Available from garden and craft supply stores, dried green moss should be soaked in water before being placed into the aquarium. Place new moss in a dry 19 L (5 gal) bucket, shake well, then discard loose debris collected at the bottom of the bucket. Next rinse several times to remove remaining fines, and soak in hot water for 20 minutes to leach out tannins. Place handfuls of soaked moss into filled tanks, letting the moss settle to the bottom. A 5–8 cm (2–3 in) layer of moss is adequate for covering the bottom of a tank.

Lighting

Overhead fluorescent room lights are adequate for blackfish; fish are less stressed if lighting is indirect and dimly diffuse. For non-reproductive fish, a 16L/8D photoperiod is adequate. I also attach a supplemental light above each tank to provide ample illumination while cleaning tanks or conditioning fish for spawning. Hang one 1.2 m (4 ft) full-spectrum fluorescent light strip with one or two bulbs directly above each tank. Sometimes I supplement ambient room light by turning on each tank fluorescent light for 3–6 hr/day. Use an automatic timer. To condition blackfish for spawning, photoperiod should simulate natural day length in the Arctic and also be synchronized with water temperature to simulate natural seasons.

Water Chemistry

Use aquarium test strips to check water chemistry between water changes. Keep ammonia levels at 0 and nitrates and nitrites at recommended levels for general fish keeping, through regular water changes. (Consult a general aquarium how-to manual.) Tapwater alkalinity levels are adequate for blackfish.

1) **Temperature** should be kept at a low room temperature of 16° C (61° F) or less, without chillers. 30° C (86° F) is lethal to blackfish. With chillers, a tank temperature of 12–15° C is ideal (54° F–59° F). If fish are to be conditioned for spawning, temperatures should duplicate natural arctic seasons, with a range of 4 °C–15 °C (39° F–59° F).
2) **pH** can be variable, excluding highly acidic or alkaline levels. A range of 6.5–7.8 is well-tolerated by blackfish.
3) **Salinity** should be low, around 0.5–2 ppt, as blackfish are freshwater teleosts.

Tank Maintenance

Water Replacement

Set up an extra tank to hold replacement water. First, fill with cold tap water. Then, for a 303 L (80 gal) tank, add 300 g (1 cup) or less synthetic sea salt dissolved in a bucket of tap water. Add one small sponge filter connected to an air line/air supply. Aerate the water for several days to remove chlorine before adding to a blackfish tank. Tank can be refilled with tapwater via a vinyl tube connected directly to the tapwater faucet.

Change about one third of the fish tank water every two weeks. (Frequency of water changes will depend on tank stocking density, amount of feed fed, and fish tank water temperature.) Use a submersible pump, with inlet covered by a foam insert, to siphon wastewater from the bottom of the blackfish tank. (Be sure to follow state agency permit stipulations for treating wastewater prior to disposing into municipal sewer drains.) Replace wastewater with aerated water from the replacement tank. Use a submersible pump kept in the replacement tank and attached to vinyl tubing to transfer fresh replacement water into the blackfish tank, or siphon between tanks. Large metal spring clamps from a hardware store are useful for holding tubing in place. I often siphon cold tap water directly into my blackfish tanks, as replacement water, without adverse effects, if one third or less of the fish tank water is being changed. (The amount of chlorine in our municipal water is not harmful to blackfish, in my experience. Of course, this varies by location, and the safest approach is to aerate to remove chlorine before using tap water.)

A fine-mesh aquarium net is useful to remove debris from tank water during water changes. Gently sweep the net through the fish tank to catch swirling debris. Visible feces and uneaten feed can also be scooped off the tank floor with a hand net or siphoned off with a hose.

Cleaning Filters and Substrate

Sponge filters should be cleaned every 4–6 wks. Disconnect the air supply, gently lift out the sponge filter, disassemble, and rinse thoroughly in the sink under cool running water to remove accumulated waste that clogs the filter and prevents proper functioning. Avoid extremely hot or cold tap water to prevent killing beneficial denitrifying bacteria in the biological filter. Periodically, disassemble all plastic filter parts and clean off mineral deposits that build up and block air flow. Over time, filters should be replaced. Replace one filter per tank at a time by squeezing out some of the dirty filter debris directly onto a new sponge filter, thus transferring beneficial denitrifying bacteria to the new filter before placing it in the tank.

Moss as substrate is difficult to clean; uneaten feed and feces accumulate in the moss and must be rinsed out or the moss should be replaced regularly. Visually check the moss for uneaten feed, which can foul the tank. Remove dirty moss, place in a plastic tub or bucket, and rinse thoroughly under tap water to decant debris. Moss degrades over time and should eventually be replaced. Due to the difficulty of cleaning moss, you may choose to use PVC tunnels on bare substrate, as hiding places for blackfish.

Stocking Density

Stocking densities of 5–7 adult fish per 303 L (80 gal) tank are ideal; conspecifics interact and are less stressed than solitary individuals. High stocking densities can be tolerated by blackfish due to their natural ability to live in crowded tundra pools and breathe atmospheric air; however, in intensive rearing systems, water quality must be carefully monitored even for hardy blackfish, to avoid disease and unexplained mortalities.

Feeding

Blackfish will not accept flake food. Feed should be frozen, lighty-thawed chironomid larvae ("bloodworms"). Adult blackfish ration is two 4 cm × 4 cm (1.5 in × 1.5 in) chunks per fish per feeding. Slightly thaw an unopened bag of frozen bloodworms by holding it briefly under hot running water; cut open the bag and empty into a glass baking dish. Be sure to wear gloves when handling feed. Let food float in the tank; blackfish can tolerate eating slightly frozen food. Feed adult blackfish every 2–3 days. Juveniles should be fed daily. Adjust amount of feed based on fish size, water temperature, and season. The warmer the water and the longer the photoperiod, the more feed blackfish will consume. During short photoperiods mimicking Arctic winter conditions, blackfish will eat less or may stop feeding altogether. Avoid overfeeding because uneaten feed fouls water and is difficult to remove from the moss substrate. Blackfish are voracious eaters during peak temperature and photoperiod–Arctic summer conditions.

I have good success feeding blackfish only *Chironomid* larvae; ideally, blackfish should be offered a variety of *Chironomid* larvae, small aquatic crustaceans, snails, and even live fish (if IACUC protocol and permits allow). I use the following live feed for diet variety and sensory stimulation for blackfish:

* live glass shrimp (*Palaeomonetes*)
* live bloodworms (Chironomidae)
* live freshwater snails (A local aquarium retailer is usually happy to donate excess, nuisance snails.)

You can also culture many live fish foods. (See the North American Native Fish Association's website and forum for helpful tips–http://forum.nanfa.org/)
Local lakes and ponds yield abundant small crustaceans such as *Daphnia* and *Chironomid* larvae, which are easily harvested with fine-mesh nets. Beware of introducing pathogens to lab aquaria through use of wild-caught food. My blackfish refuse to eat frozen adult *Artemia* (brine shrimp). Sometimes adults will accept frozen, thawed chopped silversides (*Menidia*, a marine fish).

Mortalities

Sick or diseased blackfish can be euthanized with an overdose of buffered MS-222, a fish anesthetic. Dead fish ("morts") should be removed from aquaria immediately to avoid fouling the water. Report mortalities to the IACUC, as per permit guidelines. If possible, weigh, measure, and dissect the specimen for documentation. Check external morphology for any visible trauma. A necropsy may reveal parasites, tumors, an enlarged spleen, dark liver pigmentations, or liver cysts. I experienced low-grade but persistent mortalities, especially during winter months; the most common symptoms were coelomic edema and an enlarged spleen. The life span of blackfish is not well-documented; some of my specimens were at least 5 years old, and some mortalities may have been due to natural senescence.

Blackfish Behavior

Captive blackfish exhibit interesting behavior, a trait that makes them rewarding aquarium fish. Over time, fish can become quite tame and interact with the person feeding them. When handled, they often display a stress response in which fin tips turn bright red due to peripheral blood flow. (My artificially-spawned blackfish exhibited this response during handling, at age one.)

Domesticated blackfish are often territorial, especially during feeding. Impressive fight displays include gill flaring, side-wagging, pectoral fin-fanning, and jaw-locking. An aggressor might bite another blackfish's side, gill cover, or jaws and stay latched on for many minutes. Because blackfish have small teeth, no permanent physical damage results.

On one occasion, I observed three males in three different tanks on the same day react to a visual stimulus–a fluorescent orange camera bag suspended from my camera during filming– https://www.youtube.com/watch?v=Nzb28w4LQq4. The swinging orange bag seemed to trigger a reaction by one dominant blackfish in each of three different tanks on the same day in late springtime. Territorial displays by each blackfish appeared to be directed towards the camera and also toward any tank mates that swam too close. Photoperiod and temperature had been previously manipulated for several months, to mimic Arctic seasons, and these responses may have signaled early establishment of territories in preparation for spawning, although these blackfish did not spawn.

Artificial Fertilization

Blackfish can be artificially fertilized in the lab. Prepare a batch of sterile "fish water" by boiling aquarium water or lab RO water and adding synthetic sea salt, 4 ppt. Store "fish water" in a plastic jug and aerate for 24 hours. Euthanize one ripe female blackfish with an overdose of buffered fish anesthetic such as MS-222. Rinse off any anesthetic and then collect ripe eggs by either gently squeezing them from the vent or dissecting the entire ovary of ripe eggs into a clean Petri dish. Fully ripe blackfish eggs are pale golden, transparent, 2 mm diameter, and wrinkle when placed in water. Unripe eggs look translucent, not clear, and will not fertilize. Euthanize 1–2 adult males, dissect the testes, then mince and mash testes in a clean Petri dish to release milt. You can check sperm motility by viewing a small drop of milt under a compound microscope. Use a squeeze bottle of “fish water” to wash milt onto the eggs, then very gently stir the egg/milt/water slurry to combine. Set aside for about 5 minutes to allow for fertilization, then gently wash the contents into a 1 L glass jar. Carefully fill the jar 3/4 full of “fish water” to which 1–2 drops of Methylene blue (fungicide) have been added. Add an airstone connected to an air pump; position the airstone just below the water surface, and adjust airflow to create a very gentle current over the eggs. Fertilized blackfish eggs will sink and stick together.

Hatch jars should be placed in a cool room with ambient light. I hatched a small cohort of blackfish using this method, but most embryos were deformed and died before or shortly after hatch. Dr. Trent Sutton at the University of Alaska Fairbanks suggests rearing embryos at cooler temperatures to avoid developmental deformities. I used 16° C (61° F) with resulting deformed embryos. Try using 12° C (54° F) or cooler. Carefully suction off debris and dead embryos daily; embryos are sticky and usually coated with a small amount of debris, which is OK. Change 1/3 hatch water once a day, replacing with tempered “fish water”. After hatch–on day 5 at 16°C (61° F)–larvae can remain in the hatch jars for several weeks with daily partial water changes.

Once the yolk sac is completely absorbed, larvae are fed live newly hatched brine shrimp (*Artemia*) nauplii. I use nauplii less than 24 hours old to ensure optimum nutritional content. I tried unsuccessfully to feed larvae cultured live “vinegar eels” (nematodes, *Turbatrix acetic*); blackfish larvae rejected them. Use the following method to hatch daily supplies of *Artemia* nauplii: Set up a 38 L (10 gal) glass aquarium filled with about 8 cm (3 in) tap water; place a submerged aquarium heater at the bottom of the tank. Fill a 1 L jar 3/4 full of tap water, then add 20 g (1 T) sea salt and ¼ tsp. Artemia eggs (cysts). Place a 1 L jar of tap water containing 15 ml (1T) sea salt and 1 g (¼ tsp) *Artemia* eggs (cysts) into the glass tank, making sure the tank water is well below the surface of the brine shrimp hatch jar. Add an airstone connected to an air pump; adjust to a gentle constant flow of bubbles. On the exterior of the glass tank, place a label across from the hatch jar with start time/date. Brine shrimp should hatch within 24 hrs at 27° C (80° F). Start a second *Artemia* culture in a new jar, 12 hours after the first jar, and so forth, to produce daily live feed for larval blackfish.

To harvest newly-hatched brine shrimp, remove the air stone, place the hatch jar on a counter, and cover it with a black piece of fabric that has a small hole cut on one side. *Artemia* nauplii are attracted to light and will gather near the light source where they can be suctioned off. Siphon nauplii into a fine mesh brine shrimp net (available at aquarium supply stores), rinse with tap water, then pour into the jar containing blackfish larvae. Allow blackfish to feed to excess–you can see their stomachs bulging full of orange-colored *Artemia*–and then suction out and discard remaining nauplii.

As larvae become juveniles, transfer them into glass aquaria, 38 L (10 gal) or larger, and add some washed sphagnum moss to provide hiding places. A alternative live aquatic plant is Java moss (*Taxiphyllum barbieri*; synonym: *Vesicularia dubyana*). Often sold in aquarium stores, it is an attractive bright green color and grows well under adequate lighting. As juvenile blackfish grow, they should be fed frozen-thawed or live bloodworms. Other food can include live zooplankton collected from ponds and lakes, but be cautious about introducing pathogens into enclosed systems.

Spawning Blackfish in Captivity

The following techniques are suggested for spawning Alaska blackfish in the laboratory. Reduce tank water temperature with aquarium chillers, and adjust photoperiod with full-spectrum lights set on timers to simulate natural arctic conditions. Add about 8 cm (3 in) of washed green moss to the bottom of each tank, to be used as spawning substrate. (Blackfish are believed to spawn on submerged plants, although this has not been documented.) Try conditioning ripening females with a variety of feed including chopped frozen, thawed fish (silversides, *Menidia*). Install a wireless camera to monitor spawning behavior 24 hr/day.

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