Pacific herring *Clupea pallasii* and wrack macrophytes subsidize semi-terrestrial detritivores

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ABSTRACT: The bi-directional movements of materials, nutrients and energy across the land–sea interface result in a number of ecological consequences. Migratory animals that move across ecosystems drive a number of these spatial subsidies (e.g. anadromous salmon), but many of the world's animal migrations are in decline. The cross-ecosystem reach of Pacific herring *Clupea pallasii*, a migratory, iteroparous forage fish that spawns along the coastlines of the North Pacific Ocean, has never been studied. Spawn events represent large aggregations of biomass and energy, a portion of which is transferred to intertidal and supratidal zones. Using fatty acids and stable isotopic signatures of carbon and nitrogen, we (1) traced the sources of production and (2) tested the hypothesis that herring subsidizes detritivorous, semi-terrestrial amphipods (Talitridae: *Traskorchestia* spp.), which are often abundant in intertidal and supratidal zones. Amphipods and likely dietary items (macrophytes and herring eggs) were collected from 5 beaches before and after herring spawned in Quatsino Sound, British Columbia. Combined with the use of mixing models, stable isotopes suggest that herring, in addition to brown algae and seagrass, were major sources of production during the study period. Fatty acid results suggest that brown algae is generally a major dietary resource for amphipods, but herring eggs provide important omega-3 fatty acids that are low in abundance prior to herring spawn events. Taken together, we provide corroborative evidence of a previously unknown cross-ecosystem spatial subsidy by Pacific herring. Further, because amphipods are prey for several terrestrial consumers, we also identify previously unknown marine–terrestrial linkages.

KEY WORDS: Pacific herring · Spatial subsidy · Wrack · Amphipoda · Detritivores · Fatty acids · Stable isotope

INTRODUCTION

Ranging from estuaries to rocky shores, the dynamic land–sea interface (or coastal ecotone) stretches over 1.6 million km worldwide (Burke et al. 2001). Known as spatial subsidies, bi-directional flows of nutrients, energy and material into and across this interface link marine, intertidal and terrestrial ecosystems, and are critical to biodiversity persistence and ecosystem function (Álvarez-Romero et al. 2011). Migratory animals routinely cross ecological boundaries, playing significant roles in subsidizing receiving ecosystems and influencing changes in ecosystem productivity, community structure, trophic cascades and more (Helfield & Naiman 2001, Schoenecker et al. 2004, Varpe et al. 2005, Darimont et al. 2008, Hocking & Reynolds 2011 and others). Referred to as 'endangered phenomena' (Brower & Malcolm 1991), many of the world's animal migrations have been greatly reduced in size, range or stopped altogether (e.g. passenger pigeons and plains bison; Wilcove & Wikelski 2008). Consequently, the ecological processes associated with migratory movements, including spatial subsidies, similarly diminish or cease.

On the Pacific coast of North America, spatial subsidies generated by spawning anadromous Pacific

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salmon have been intensively studied (e.g. Reimchen 2000, Darimont et al. 2008, Hocking & Reynolds 2011 and others) within the ecological context of high fisheries exploitation and declining populations (Darimont et al. 2010). But the movement of animals, nutrients and material across habitats and ecosystems is ubiquitous (Polis et al. 1997), and there are other spatial subsidies at the land–sea interface that are understudied. Here we identify nearshore and intertidal spawning Pacific herring *Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847 as a provider of spatial subsidies to intertidal and possibly terrestrial ecosystems. Their sister species, Atlantic herring, is responsible for the largest known single population energy flux on the planet (Norwegian spring-spawning Atlantic herring; Varpe et al. 2005), and several species of anadromous Atlantic clupeids subsidize freshwater ecosystems (e.g. Garman & Macko 1998, Walters et al. 2009). Despite suggestions in the literature that Pacific herring, an inshore spawning forage fish, may be important prey for terrestrial consumers and act to subsidize terrestrial systems (Willson et al. 1998), Pacific herring have never been investigated for their cross-ecosystem interactions, apart from their relationship with the haul-out locations of Steller sea lions (Womble et al. 2009).

Pacific herring is a highly abundant, cornerstone species in the coastal foodwebs where they occur (sensu Willson et al. 1998). Each year, fish migrate to shallow subtidal and intertidal zones to spawn, with most Pacific herring spawning from February to April in British Columbia (BC), Canada. At spawn events, aggregations of iteroparous adult fish broadcast milt into the water column and deposit adhesive eggs onto subtidal and intertidal substrates. Combined, adult spawners and spawn products represent a pulsed subsidy to coastal ecosystems (Willson & Womble 2006) that is important for a diversity of species, including intertidal and potentially terrestrial organisms.

Intertidal and supratidal zones receive a variety of spatial subsidies, and those with low *in situ* productivity are often reliant on allochthonous sources of production (Colombini et al. 2003). From productive coastal marine ecosystems, shorelines receive wrack (drift macrophytes) in quantities that range from 10s to 1000s of kg m^{-2} each year (Polis & Hurd 1996), which wash up on a variable but relatively continuous basis. Other subsidies, including animal drift such as pinniped carcasses (Polis & Hurd 1996) and the products of beach spawning fishes (mainly eggs), including capelin (DeBlois & Leggett 1993) and likely Pacific herring, represent more spatiotemporally constrained or 'pulsed' subsidies to intertidal and supratidal zones. Receiving these subsidies of wrack, carrion and fish eggs is a large community of intertidal and supratidal predators, scavengers and detritivores, including amphipods (DeBlois & Leggett 1993, Polis et al. 2004). Talitrid amphipods, commonly known as sand hoppers or beach fleas, are abundant semi-terrestrial detritivores (Koch 1990). As major consumers of wrack in intertidal ecosystems (Griffiths et al. 1983, Lewis et al. 2007), amphipods can play a significant role in the conversion of energy and material. In turn, amphipods are prey for a diversity of intertidal (e.g. shore crabs; Lewis et al. 2007) and terrestrial predators (e.g. black bears; Ellis & Wilson 1981). Consequently, they represent a potential indirect trophic link between wrack, Pacific herring and terrestrial ecosystems.

Across 5 low *in situ* productivity beaches that receive variable Pacific herring spawn, we used fatty acids (FAs) and stable isotopes to investigate how the sources of production supporting talitrid amphipods (*Traskorchestia traskiana* Stimpson, 1857 and *T. georgiana* Bousfield, 1958) varied in response to herring spawn in Quatsino Sound, BC. Because differences in the isotopic composition of the diet are typically assimilated in the tissues of consumers, stable isotopes are commonly used in the study of spatial subsidies (Fry 2006). As few animals can synthesize polyunsaturated fatty acids (PUFAs) *de novo* and the majority are acquired from dietary sources (Brett & Muller-Navarra 1997), PUFAs are increasingly used in dietary studies, both qualitatively and quantitatively as trophic tracers (Budge et al. 2006). Previously used to trace the contribution of wrack to amphipods (Crawley et al. 2009) and Pacific herring in the diets of white-winged scoter *Melanitta fusca* and surf scoter *M. perspicillata* (Anderson et al. 2009), the combined approach of FAs and stable isotopes as trophic tracers provides an opportunity to independently assess dietary contributions. In this study, FAs and stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were used to (1) trace sources of production and (2) test the hypothesis that Pacific herring spawn provides a spatial subsidy to semi-terrestrial amphipods.

MATERIALS AND METHODS

Study area

Quatsino Sound, located off the northwestern tip of Vancouver Island, BC, was selected as a study region due to the presence of a small but reliable Pacific herring spawn and somewhat intact coastal ecosystems,

with fragmented old-growth forests and large *Macrocystis integrifolia* forests and seagrass meadows in the nearshore. Study sites consisted of 5 beaches (100 m wide) located in Quatsino Sound, which were classified based on the presence and relative density of Pacific herring eggs: high, medium and low egg densities (hereafter High, Medium and Low beaches), and Control 1 and Control 2 with no eggs (Fig. 1, see Fig. 2). Substrates on all beaches were mixed, consisting of sand, gravel, cobble and boulder. With the exception of one beach site (Medium), beaches were exposed to open-ocean swell, and *M. integrifolia* sporophytes formed large forests in the nearshore. At all beaches, large *Phyllospadix serrulatus* meadows grew in the nearshore subtidal and low intertidal. Mid and high intertidal zones at all beaches were characterized by relatively low *in situ* productivity (i.e. low macrophyte cover and densities of macroinvertebrates), with the exception of the boulder beach (Low), where *Fucus* spp. was dominant in the mid intertidal zone. In the high and supratidal zones, variable quantities of wrack macrophytes support a diverse, wrack-reliant community. At our 5 sites, *Traskorchestia traskiana* and *T. georgiana* were the most abundant wrack-inhabiting taxa, with a mean dry biomass of 27.7 g

m^{-2} ± 10.9 SE in the high (3 to 4 m) and supratidal (>4 m) zones (C. H. Fox unpubl. data).

Pacific herring spawn annually in Quatsino Sound in March or April. In 2011, roughly 1000 metric tons of Pacific herring spawned in the nearshore subtidal and intertidal zones (Fisheries & Oceans Canada 2011). However, yearly monitoring is incomplete in this region and estimates of adult spawner biomass are not intended for quantitative use. Spawning at study sites began on March 21 and continued until at least March 23; no milt was observed at any study sites after this date (C. H. Fox pers. obs.).

Sampling

Presence of spawn was assessed on all 5 beaches by visual inspection of the water for milt, aggregations of Pacific herring and attached eggs on nearshore and intertidal substrates, including blades of *Macrocystis integrifolia*, *Phyllospadix* spp. and other macrophytes. Intertidal zones were searched on foot. On 29 and 31 March 2011, beaches where eggs were found or where milt had been observed (High, Medium and Low beaches) were surveyed using 25 randomly distributed 0.25 m^2 quadrats over a 100 m portion of beach in order to measure egg density. Along 5 perpendicular transects, 5 quadrats were randomly assigned to stratified tidal heights in the low (1–2 m), mid (2–3 m) and high (3–4 m) intertidal, in addition to 2 quadrats laid along the fresh (within the 3 to 4 m zone) and older, degraded supratidal wrack lines (>4 m, LLW). Percent cover of eggs was visually estimated and converted to number of eggs using a standard egg radius (r = 0.74 mm; Alderdice & Hourston 1985). Egg depth (number of layers) was randomly counted at 5 locations per quadrat and the resulting average used as a multiplication factor for percent egg cover, to estimate total eggs per quadrat.

Fresh wrack biomass was measured at all 5 locations on 2, 3 and 5 May 2011, 0 to 3 d following a moderate storm. Although wrack is continuously washed onto beaches, storm events often load beaches with significant quantities. On each beach, all wrack found within 3 randomly distributed 0.25 m^2 quadrats in the fresh wrack line was sorted and identified to the lowest possible taxonomic group. Wrack was immersed in seawater, shaken and weighed. Dry weights of a subset of all species and groups were collected after drying at 60°C for 24 h, and the ratio of wet:dry mass was used to generate dry mass estimates for all samples.
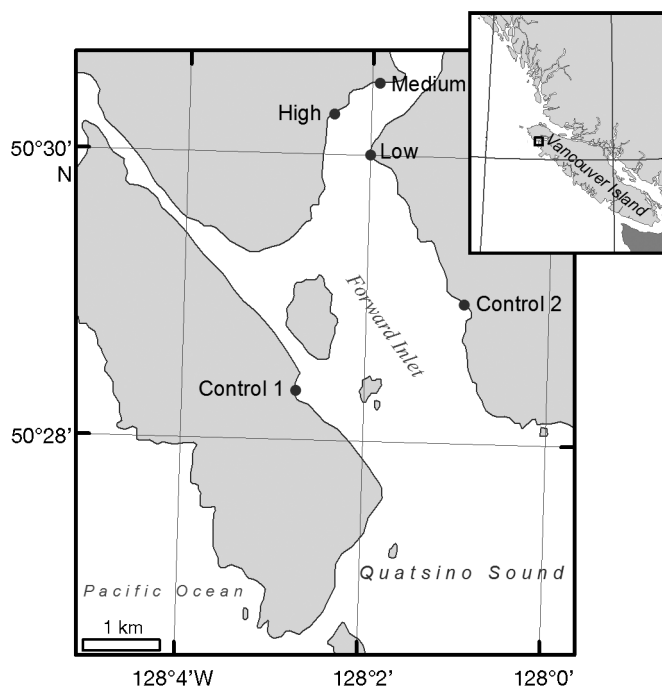

Fig. 1. Study beach sites in Quatsino Sound, British Columbia: High, Medium and Low (referring to Pacific herring *Clupea pallasii* egg density), and Control 1 and 2 (no eggs), in the intertidal zone. Beaches were sampled before (March) and after (April) Pacific herring spawn events in 2011

Stable isotopes

Samples for stable isotope analysis were collected within a 100 m horizontal stretch of beach study site, from the subtidal through to the high intertidal zone. Depending on the species, samples were collected from a restricted 1 m tidal height width that represented the highest concentration of that species. Macrophyte tissues from live, intact attached plants in the nearshore and intertidal were collected with 3 blades from 3 plants growing nearby, constituting one sample (n = 5 samples per beach, per sampling period). Macrophyte species collected were those considered to be major components of the wrack and likely sources of production for amphipods (*Phyllospadix serrulatus*, *Macrocystis integrifolia*, *Fucus* spp., *Callithamnion* spp., and *Ulva lactuca* when available). Macrophytes were scraped with a razor blade or combed with tweezers if foliose, rinsed with distilled water and frozen at −20°C. Live amphipods, consisting of a mix of *Traskorchestia georgiana* and *T. traskiana*, were collected using a 10 cm sand core in the fresh wrack line, kept cool during 1 to 2 h of transport, and rinsed with distilled water. Both species were combined for stable isotope and FA analysis due to difficulties associated with identification of live individuals (as required for FA analysis given our field conditions) under a dissecting microscope. Multiple individuals (~4 to 8) from one sand core were pooled per sample (n = 10 per beach, per sampling period) before being frozen at −20°C. All samples were collected before and after the Pacific herring spawn, on 7, 8, 13, 16, 17 and 20 March and 11, 12, 13, 15 and 16 April 2011, with exception of the eggs of Pacific herring, which were collected from 2 of the 3 spawn beaches on 29 March. Eggs from the third beach were heavily fouled and were not collected. Eggs were processed using the same methods as amphipods.

Samples were dried for 48 h at 60°C and were then ground into a fine powder using a Wig-L-Bug amalgamator. Sub-samples (~1 to 3 mg) were packaged in tin capsules and analyzed for total and isotopes of nitrogen and carbon at the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrophotometer (Sercon). Natural abundances of ^{15}N (δ^{15}N) and ^{13}C (δ^{13}C) are expressed as deviation from the standards (atmospheric N_2 and Pee Dee Belemnite) in parts per thousand (‰) calculated by $(R_{sample}/R_{standard} - 1) \times 1000$, where R is the heavy:light isotope ratio. Higher values of δ^{15}N and more positive values of δ^{13}C con-

tain higher proportions of the heavy isotope relative to lighter isotopes. Post-hoc lipid corrections were applied to δ^{13}C animal tissue samples (Pacific herring eggs and amphipods) using C:N ratios, following Post et al. (2007).

Fatty acids

Concurrent with stable isotope sampling dates, 3 amphipod samples (~10 individuals each) were collected from each beach in periods before and after the spawn. Herring eggs were collected from the High beach (n = 3) and macrophytes were sampled from High, Medium and Low beaches in the pre-spawn period, in part due to accessibility and availability of unfouled sporophytes. Given the qualitative FA macrophyte analysis but also the previous finding of low macrophyte FA variation at spatial scales greater than this study (Crawley et al. 2009), there was little rationale for sampling macrophytes more extensively. After collection, all samples were kept cool during 1 to 2 h transport. Macrophytes were scraped with razor blades or combed with tweezers before all samples, including amphipods and herring eggs (1 g wet wt), were rinsed 3 times with distilled water and immersed in HPLC-grade chloroform with 0.01% butylated hydroxytoluene (BHT) as an antioxidant (Budge et al. 2006). Samples were sealed with Teflon tape and frozen at −20°C for 1 to 2 mo until being transported to the lab. Once in the lab, samples were flushed with N_2, re-sealed with Teflon tape and frozen at −80°C until FA analysis.

FAs were extracted following established protocols (Parrish 1999, Kainz et al. 2004). Briefly, samples were sonicated, vortexed and centrifuged 3 times in a 4:2:1 chloroform:methanol:distilled water solution that took place on ice and under N_2 gas. Organic layers were pooled after each extraction round, their volume standardized, flushed with N_2 and stored at −80°C. FAs were esterified to methyl esters using 14% BF_3-CH_3OH at 85°C for 1 h and analyzed using a gas chromatograph (Varian CP-3800) supplied with a Supelco 2560 capillary column (100 m, 0.25 mm inside diameter, 0.2 µm thickness) and a flame ionization detector. We identified individual methyl esters by comparing retention times against a commercial standard (37-component FAME mix (Suppleco 47885-U and Nu-Chek Prep GLC standard 487). Unmethylated tricosonic acid (23:00) was used as an internal standard to assess the efficiency of FA extraction and esterification and total FA yields were adjusted accordingly. FAs are expressed in X:Yn-Z

notation where X is the number of carbon atoms, Y is the number of double bonds, and Z is the location of the bond from the methyl terminus of the molecule.

The bulk of the FA analysis focuses on PUFAs. Marine fish contain high proportions of essential 'omega-3' PUFAs such as docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) because their food webs are ultimately supported by phytoplankton, which synthesize these PUFAs in high concentrations (Dalsgaard et al. 2003). In contrast, macroalgae contain lower proportions of PUFA than secondary consumers and are expected to be more enriched in 18-Carbon PUFA such as 18:2n-6 (Alfaro et al. 2006). Differences in the sources of production (herring eggs versus wrack) are therefore expected to change the composition and overall proportions of PUFA in amphipods. In contrast, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are less likely to be informative as trophic tracers in this particular system because all animals synthesize them, and dietary signatures can be obscured by differences in *de novo* synthesis between animals and their prey (Dalsgaard et al. 2003).

Statistical analysis

Analysis of percent FA information was performed on PUFAs expressed as percent of total FAs. Using PRIMER v6.0, we constructed 2 Bray–Curtis dissimilarity matrixes (Bray & Curtis 1957) and visualized these matrixes using non-metric multidimensional scaling (NMDS). The dissimilarity matrixes and NMDS were separately conducted using (1) PUFAs of amphipods and potential sources of production and (2) PUFAs of amphipods at beaches before and after the spawn. Ordination stress values between 0 and 1 indicate goodness-of-fit, with lower values indicating better fit. An analysis of similarities (ANOSIM) was performed on PUFAs of amphipods to test for significant differences between clusters identified using NMDS. A similarity percentage (SIMPER) analysis was used to identify the individual PUFAs that contributed to the observed groupings. Once identified, we performed 2 related analyses on 2 individual PUFAs (EPA and DHA), expressed as a percent of total FAs (after arcsine transformation) and raw concentration (mg g^{-1} wet wt). A general linear mixed model (GLMM) specified with a repeated measures design (before/after), beach site as random effect and herring egg presence (binary presence or absence) as a fixed effect was used to determine whether the presence of herring had a significant influence on percent total fatty acids and raw concentrations of EPA and DHA. This analysis was followed by 1-way ANOVAs that afforded the ability to examine within-beach differences before and after the spawn.

For amphipod stable isotopes, we used a similar approach with GLMMs (repeated measures design and beach site as a random effect) to determine whether the presence of herring eggs (fixed effect) influenced δ^{13}C and δ^{15}N values followed by 1-way ANOVAs to compare differences in mean isotopic values at beaches in periods before and after the spawn using SPSS v20. We also used a Bayesian mixing model (MixSIR v1.0; Semmens & Moore 2008) to estimate the probability distributions for the proportional contribution of each source of production to amphipods on all 5 beaches in periods before and after the spawn. Informative priors were not specified. Stable isotope signatures of a consumer relative to its diet can differ due to differential digestion and fractionation by the consumer (McCutchan et al. 2003). MixSIR, like other mixing models, requires specification of enrichment factors, sometimes referred to as consumer-diet discrimination values. Model outcomes have been shown to be sensitive to the enrichment factor used, and the use of generalized enrichment factors has been questioned (Mancinelli 2012 and others). For the MixSIR models, we used generalized isotopic enrichment factors from McCutchan et al. (2003) for aquatic consumers (δ^{13}C: +0.40 ± 1.20 SD; δ^{15}N: +2.30 ± 1.61) modified for standard deviation reporting as required by MixSIR for all but 2 species. We acknowledge that these generalized enrichment factors have not been demonstrated for *Traskorchestia* spp.

Isotopic enrichment factors for *Macrocystis integrifolia* and Pacific herring eggs were determined during a 2 wk, lab-based amphipod feeding trial. In 2 self-draining tanks with dripping seawater and a daily wash with freshwater (to rinse salt build up away and provide freshwater for amphipods), a mix of clean beach gravel and sand was used as habitat for ~200 amphipods (mix of *Traskorchestia traskiana* and *T. georgiana*) per tank. Either fresh *M. integrifolia* blades or herring eggs were placed into each tank and left to be consumed and/or degrade. Algae and eggs were periodically added at a rate that minimized rot but also allowed unrestricted consumption by amphipods. Dead amphipods were removed daily. By comparing δ^{13}C and δ^{15}N levels in amphipods sampled prior to the experiment and after 2 wk, we generated δ^{13}C and δ^{15}N isotopic enrichment factors

for amphipods fed *M. integrifolia* (+2.58 ± 1.21 SD and +4.34 ± 0.65 respectively) and for those fed Pacific herring eggs (+3.90 ± 0.38 and −1.91 ± 0.38 respectively). We recognize that these values differ from McCutchan et al. (2003) and others (e.g. Vander Zanden & Rasmussen 2001) and interpret MixSIR model output cautiously, given that we have used a combination of generalized and consumer-specific enrichment factors. Further, given the length of the feeding trial, it is unlikely that *Traskorchestia* spp. reached equilibrium with their resource; however, this 2 wk period approximates the time frame for *Traskorchestia* spp. to have access to herring eggs in the wild.

In 3 instances, we used stable isotope values from proximal beaches as dietary sources in the MixSIR models. At Low beach, eggs were fouled and we used the average stable isotopic values from the remaining 2 spawn beaches for model input. Few macrophyte species grow at Medium beach and 2 macrophyte species were not present. Two adjacent beaches with large *Macrocystis integrifolia* forests and high densities of *Fucus* spp. were likely sources of wrack; average stable isotopic values from High and Low beaches were used as model input for *M. integrifolia* and *Fucus* spp. on Medium beach.

RESULTS

Pacific herring spawned on 3 of 5 beaches, resulting in intertidal egg densities (mean ± SE) that were categorized as High, Medium and Low; 1.07 ± 1.01, 0.30 ± 0.15 and 0.02 ± 0.01 million eggs m^{-2}, respectively, in the fresh wrack line (3 to 4 m zone, where amphipod densities tend to be highest) (Fig. 2). On each beach, eggs had a bi-modal distribution, with highest densities in the low (1 to 2 m) and the fresh wrack line (Fig. 2).

Wrack loading and composition at the 5 beaches were variable, but overall, seagrasses, *Macrocystis integrifolia* and *Fucus* spp. were dominant components of the wrack (Fig. 3). For seagrasses, we could not readily distinguish between *Zostera marina* and *Phyllospadix serrulatus* due to the occasional longitudinal tearing of *Z. marina* blades and the poor condition of specimens that washed up following the storm event. However, *Z. marina* is not present at our study sites or
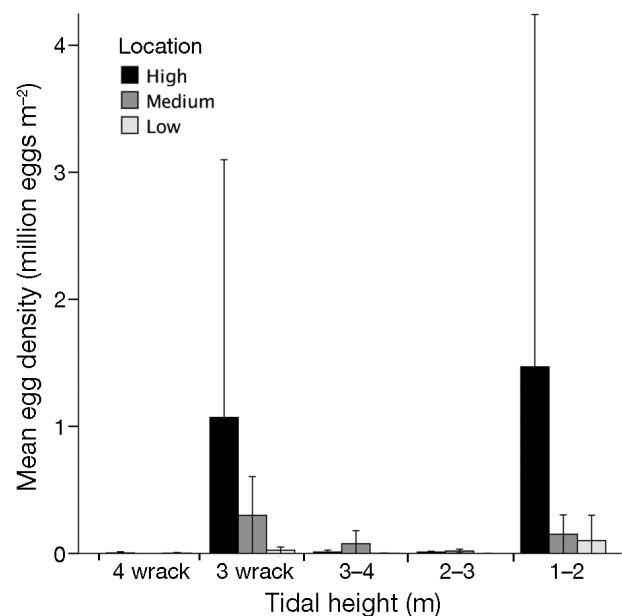

Fig. 2. *Clupea pallasii*. Mean (±SE) density of Pacific herring eggs on 3 beaches (High, Medium, Low) in Quatsino Sound, British Columbia (29 and 31 March 2011). Random stratified quadrat sampling was in the 1–2, 2–3 and 3–4 m tidal height zones, in addition to the fresh (3 wrack) and older wrack lines (4 wrack)
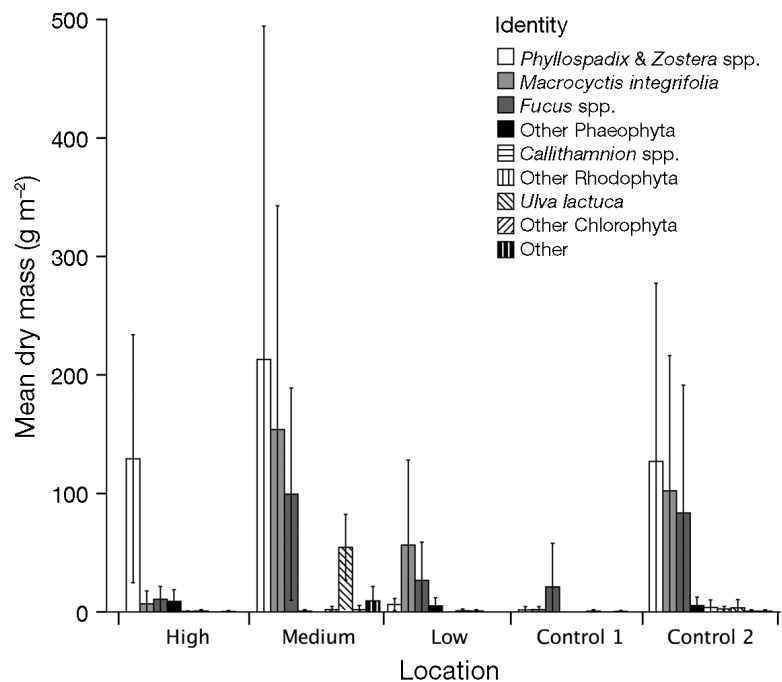

Fig. 3. Mean (±SE) dry mass (g m^{-2}) composition of fresh wrack macrophytes and other (e.g. Bryozoa and Porifera) at 5 beaches (High, Medium, Low, Control 1 & 2) in Quatsino Sound, British Columbia, excluding woody debris. Samples were collected 0 to 3 d following a moderate storm on 2 May 2011. See Fig. 1 for site details

adjacent areas, instead it grows in more sheltered waters several kilometers away. *P. serrulatus* is a dominant macrophyte at our study beaches and throughout the region; we conservatively estimate from identifiable subsamples that most (>75%) seagrass wrack was *P. serrulatus*.

Fatty acids

In amphipods, 20:4n-6, 20:5n-3 and 22:6n-3 were the most abundant PUFAs (Table 1). The 2 brown algae (*Macrocystis integrifolia* and *Fucus* spp.) were characterized by high relative amounts of 20:4n-6 (12.2 ± 0.8 [mean ± SE] and 11.5 ± 0.8) and 16:0 (12.3 ± 1.0 and 14.2 ± 0.3). Relative to other sources, *Phyllospadix serrulatus* was characterized by high relative amounts of 18:3n-3 (47.4 ± 1.4), 20:0 (2.8 ± 0.4) and 22:0 (6.2 ± 0.7); *Ulva lactuca* by high 18:2n-6 (13.2 ± 3.6) and low 20:5n-3 (3.4 ± 0.7); and *Callithamnion* spp. by high 16:1n-7 (6.7 ± 1.3), 20:5n-3 (40.1 ± 1.0) and, unlike other macrophytes that all had trace quantities of 22:6n-3, by a small amount of 22:6n-3 (2.0 ± 1.0; Table 2). Unlike macrophytes, Pacific herring eggs were characterized by high relative amounts of 16:0 (39.5 ± 0.5), 22:6n-3 (16.2 ± 1.2) and low 20:2n-6 (trace) (Table 2). An abundant PUFA in *P. serrulatus* spp., 18:3n-3 was present in very low amounts in amphipods (Tables 1 & 2). The amount of 22:6n-3 was relatively low but significantly variable on certain beaches in periods before and after the spawn in amphipods and is a characteristic PUFA of Pacific herring eggs.
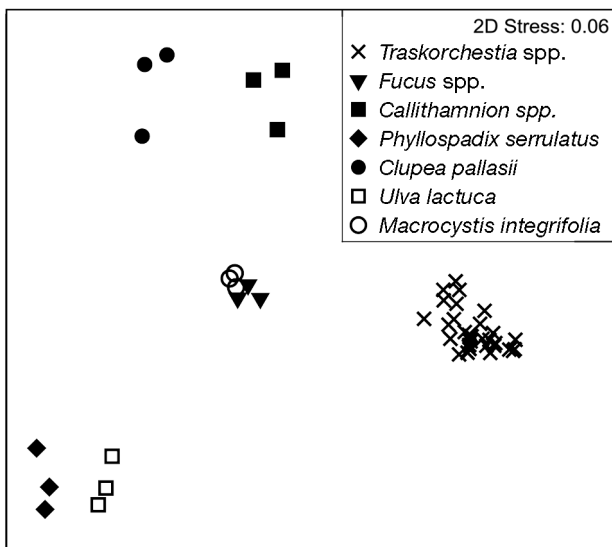
Using NMDS ordination to semi-quantitatively examine dissimilarities in consumer and food source, PUFAs revealed that most food items and consumers clustered

Table 1. Fatty acid composition (mean % total fatty acids ± SE) of amphipods (*Traskorchestia* spp.) at 5 beaches with varying Pacific herring *Clupea pallasii* egg densities (High, Medium, Low and 2 Control beaches) in Forward Inlet, Quatsino Sound, British Columbia, in periods pre- and post-Pacific herring spawn in 2011. Fatty acid values below limits of detection given as (−)

Fatty acid	High Pre-spawn	High Post-spawn	Medium Pre-spawn	Medium Post-spawn	Low Pre-spawn	Low Post-spawn	Control 1 Pre-spawn	Control 1 Post-spawn	Control 2 Pre-spawn	Control 2 Post-spawn
14:0	11.1 ± 0.5	10.1 ± 0.3	9.9 ± 0.7	9.7 ± 1.1	10.9 ± 0.3	10.7 ± 0.1	10.5 ± 0.2	9.3 ± 0.8	9.4 ± 0.2	8.1 ± 0.4
14:1	12.0 ± 0.6	9.4 ± 0.2	12.6 ± 0.4	10.1 ± 0.9	13.5 ± 0.6	11.9 ± 0.2	11.5 ± 0.3	10.3 ± 1.0	10.3 ± 0.6	7.4 ± 0.3
15:0	−	−	−	−	−	−	−	−	−	−
15:1	−	−	−	−	−	−	−	−	−	−
16:0	16.7 ± 0.3	16.5 ± 0.0	16.2 ± 0.5	17.2 ± 0.5	16.6 ± 0.1	17.4 ± 0.1	16.2 ± 0.0	16.3 ± 0.5	16.8 ± 0.4	16.0 ± 0.3
16:1n-7	15.8 ± 0.8	13.0 ± 0.3	16.9 ± 0.1	14.5 ± 0.8	17.9 ± 0.3	16.8 ± 0.7	14.5 ± 0.1	14.6 ± 0.4	15.5 ± 0.4	12.3 ± 0.2
17:0	0.1 ± 0.0	0.1 ± 0.0	−	−	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
17:1	0.1 ± 0.0	0.1 ± 0.0	−	−	0.1 ± 0.0	−	−	−	0.1 ± 0.0	−
18:0	1.2 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.0	1.7 ± 0.1
18:1n-9	26.9 ± 1.3	30.3 ± 0.3	28.8 ± 2.2	28.7 ± 2.0	26.2 ± 0.6	27.9 ± 1.4	29.6 ± 0.5	31.1 ± 1.0	29.7 ± 1.7	33.4 ± 1.1
18:2n-6	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.8 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
20:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
20:1	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	1.7 ± 0.1	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.4 ± 0.2	1.5 ± 0.1	2.1 ± 0.1
18:3n-3	1.8 ± 0.0	1.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	1.5 ± 0.2	1.2 ± 0.0	1.3 ± 0.1
21:0	−	−	−	−	−	−	−	−	−	−
20:2n-6	0.6 ± 0.2	0.5 ± 0.0	0.6 ± 0.2	0.5 ± 0.3	0.5 ± 0.1	0.3 ± 0.1	1.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
22:0	−	0.1 ± 0.0	−	0.1 ± 0.0	−	−	−	−	−	0.1 ± 0.0
20:3n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:1n-9	0.1 ± 0.1	0.1 ± 0.0	−	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
20:3n-3	0.3 ± 0.2	0.3 ± 0.0	−	−	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
23:0	−	−	−	−	−	−	−	−	−	−
20:4n-6	6.6 ± 0.1	6.0 ± 0.3	6.2 ± 0.5	6.8 ± 0.7	5.9 ± 0.2	6.1 ± 0.2	5.8 ± 0.2	6.9 ± 0.7	6.9 ± 0.1	9.2 ± 0.5
22:2n-6	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
24:0	−	−	−	−	−	−	−	−	−	−
20:5n-3	2.5 ± 0.1	4.3 ± 0.1	2.6 ± 0.2	4.2 ± 0.4	2.0 ± 0.2	2.2 ± 0.2	2.8 ± 0.1	3.0 ± 0.3	2.8 ± 0.1	4.0 ± 0.3
24:1n-9	0.1 ± 0.0	−	−	0.1 ± 0.0	0.1 ± 0.0	−	−	−	−	0.1 ± 0.0
22:6n-3	0.4 ± 0.0	2.1 ± 0.3	0.4 ± 0.2	1.5 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.6 ± 0.0

Table 2. Fatty acid composition (mean % total fatty acids ± SE) of potential food sources for talitrid amphipods (*Traskorchestia* spp.). Samples were collected from 3 beach sites in Quatsino Sound, British Columbia in 2011. Fatty acid values below the limits of detection are given as (–)

Fatty acid	Magnoliophyta Potamogetonales *Phyllospadix serrulatus*	——Phaeophyta —— Laminarials *Macrocystis integrifolia*	Fucales *Fucus* spp.	Rhodophyta Ceramiales *Callithamnion* spp.	Chlorophyta Ulvales *Ulva lactuca*	Chordata Clupeiformes *Clupea pallasii* (eggs)
14:0	0.5 ± 0.2	2.7 ± 0.6	7.1 ± 0.9	3.6 ± 0.3	1.0 ± 0.2	0.2 ± 0.2
14:1	0.3 ± 0.3	–	–	–	–	–
15:0	0.4 ± 0.0	–	0.1 ± 0.1	0.2 ± 0.0	–	–
15:1	–	–	0.1 ± 0.1	–	–	–
16:0	17.1 ± 1.4	12.3 ± 1.0	14.2 ± 0.3	17.9 ± 1.6	24.7 ± 2.6	39.5 ± 0.5
16:1n-7	1.9 ± 0.5	1.7 ± 0.4	1.4 ± 0.1	6.7 ± 1.3	1.7 ± 0.3	4.3 ± 0.3
17:0	0.5 ± 0.3	1.6 ± 0.3	2.1 ± 0.2	0.7 ± 0.2	–	–
17:1	–	0.1 ± 0.2	0.7 ± 0.7	0.3 ± 0.3	0.3 ± 0.2	–
18:0	2.1 ± 0.3	1.3 ± 0.5	0.9 ± 0.1	1.9 ± 0.4	1.0 ± 0.2	4.9 ± 0.2
18:1n-9	6.1 ± 2.1	5.5 ± 0.3	11.7 ± 0.2	10.9 ± 2.4	9.0 ± 4.0	14.4 ± 2.4
18:2n-6	6.3 ± 0.2	5.4 ± 0.2	9.2 ± 0.5	5.2 ± 0.8	13.2 ± 3.6	3.4 ± 2.9
20:0	2.8 ± 0.4	–	0.1 ± 0.1	0.1 ± 0.0	–	–
18:3n-6	0.1 ± 0.1	0.7 ± 0.6	1.0 ± 0.0	0.5 ± 0.0	1.7 ± 0.3	–
20:1	–	–	0.4 ± 0.2	0.2 ± 0.1	–	–
18:3n-3	47.4 ± 1.4	10.0 ± 0.5	6.8 ± 0.5	1.2 ± 0.2	24.3 ± 1.4	1.6 ± 0.6
21:0	–	–	–	–	–	–
20:2n-6	1.0 ± 0.3	26.2 ± 3.0	14.5 ± 1.2	2.5 ± 0.4	18.1 ± 2.6	–
22:0	6.2 ± 0.7	–	0.0 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	–
20:3n-6	–	0.2 ± 0.2	1.0 ± 0.1	0.8 ± 0.2	0.1 ± 0.1	–
22:1n-9	–	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.9 ± 0.5
20:3n-3	–	–	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	–
23:0	–	–	–	–	–	–
20:4n-6	0.2 ± 0.2	12.2 ± 0.8	11.5 ± 0.8	4.1 ± 0.5	0.5 ± 0.1	0.3 ± 0.3
22:2n-6	–	0.6 ± 0.3	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	–
24:0	2.5 ± 0.3	–	–	0.2 ± 0.0	–	–
20:5n-3	4.7 ± 1.1	19.2 ± 0.6	15.2 ± 0.8	40.1 ± 1.0	3.4 ± 0.7	14.3 ± 0.4
24:1n-9	–	–	0.6 ± 0.3	–	–	–
22:6n-3	–	–	–	2.0 ± 0.1	–	16.2 ± 1.2

separately. Amphipods were distinct from their food sources and demonstrated a high similarity to brown algae (*Macrocystis integrifolia* and *Fucus* spp.), which were closely clustered (Fig. 4). Other food sources were individually clustered along the *y*-axis, with Pacific herring eggs and *Phyllospadix serrulatus* being the most dissimilar (Fig. 4).

A second NMDS ordination was used to further examine the relationships between PUFAs of amphipods on all beaches in periods before and after a spawn resolved 2 clusters; one cluster consisted of amphipods from High and Medium beaches in the

Fig. 4. NMDS ordination of polyunsaturated fatty acids of talitrid amphipods (*Traskorchestia* spp.) and potential sources of production (trophic resources) from 5 beaches in Quatsino Sound, British Columbia, in 2011. Stress values <0.1: excellent representation of the data
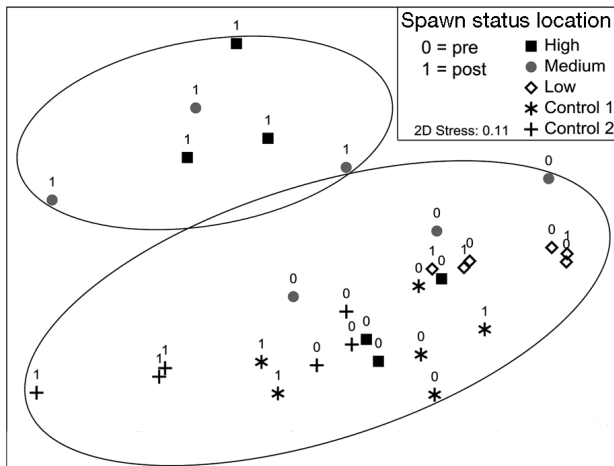
Fig. 5. NMDS ordination of polyunsaturated fatty acids of talitrid amphipods (*Traskorchestia* spp.) in periods pre- and post-spawn of Pacific herring *Clupea pallasii* at 5 beaches (High, Medium, Low spawn and 2 Controls) in Quatsino Sound, British Columbia, in 2011 (n = 3 per beach). Ovals: visually identified clusters. Stress values between 0.1 and 0.2: acceptable representation of the data with some possibility of false inferences

post-spawn period only, and the second cluster consisted of all remaining beaches (Fig. 5). Differences between the 2 clusters were significant (ANOSIM; R = 0.61, p = 0.001) and average dissimilarity between the 2 groups was moderate (SIMPER; average dissimilarity = 19.8%). The top 6 FAs contributing to this dissimilarity, in decreasing order, were: 20:5n-3 (29.7%), 22:6n-3 (25.5%), 20:4n-6 (19.2%), 18:3n-3 (9.5%), 18:2n-6 (5.8%) and 20:3n-3 (3.8%).

EPA (20:5n-3) and DHA (22:6n-3) contributed the most to the observed differences between the 2 clusters (Fig. 5). DHA is the most abundant PUFA in eggs of Pacific herring (16.2 ± 1.2%) and, with the excep-

tion of low amounts in *Callithamnion* spp. (2.0 ± 0.2%; Table 2), was only found in trace concentrations in macrophytes and pre-spawn amphipods (Tables 2 & 3). Using GLMMs, the presence of herring eggs was found to have a significant influence on mean percent (df = 1,14, F = 18.8, p = 0.002) and raw (df = 1,14, F = 6.4, p = 0.024) DHA concentrations. Differences in the mean percent DHA in amphipods in periods before and after the spawn were significant on High, Medium, Low and Control 2 beaches (ANOVA, p = 0.001, 0.010, 0.009, 0.040, respectively) but not at Control 1 (p = 0.355; Table 3, Fig. 6A). However, mean percent DHA was much larger at High (4×) and Medium (3×) beaches post-spawn, whereas differences on the Low and Control 2 beaches were marginal and largely attributable to abundance changes in other FA proportions, as evidenced by a lack of significant differences in raw DHA concentrations (Low: p = 0.142, Control 2: p = 0.451; Table 3, Fig. 6A,B). Significantly larger raw DHA concentrations were only detected on the High and Medium beaches (High: p = 0.015, Medium: p = 0.021; Table 3, Fig. 6B).

Using a GLMM, the presence of herring eggs was found to have a significant influence on mean percent EPA (df = 1,14, F = 21.2, p < 0.001) but not raw EPA concentrations (df = 1,14, F = 2.2, p = 0.160). Using 1-way ANOVAs to compare between beach differences before and after the herring spawn, significant increases in mean percent EPA were detected on the High and Medium beaches in addition to Control 2 (High: p = 0.001, Medium: 0.037, Control 2: p = 0.010; Table 3, Fig. 7A). However, significant increases in raw EPA concentrations (mg g^{-1} wet wt) were only detected on the High beach (p = 0.010) and not Control 2 (p = 0.738), likely indicating that this difference is attributable to decreases in other FAs and not changes in EPA (Fig. 7B, Table 3).

Table 3. Results from 1-way ANOVAs on 5 beaches in periods before and after a Pacific herring spawn for proportional (arcsine-transformed percent total fatty acid) 22:6n-3 (docosahexaenoic acid; DHA), total 22:6n-3 (mg g^{-1} wet wt), δ^{15}N and δ^{13}C for talitrid amphipods (*Traskorchestia* spp.) on 5 beaches in Quatsino Sound, British Columbia. Significant values in **bold** (p < 0.05). All numerator df = 1; denominator df are reported

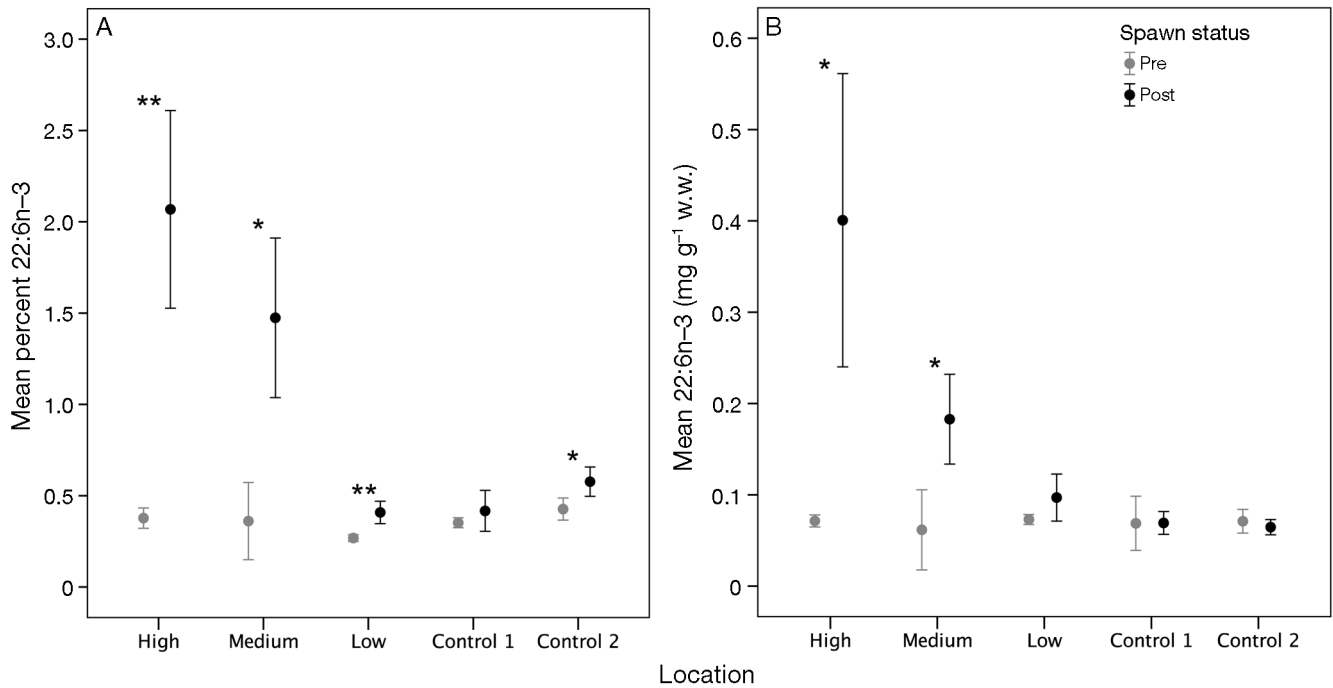| Beach	Percent total 22:6n-3 (%)			Total 22:6n-3 (mg g^{-1} wet wt)			Percent total 20:5n-3 (%)			Total 20:5n-3 (mg g^{-1} wet wt)			δ^{15}N			δ^{13}C		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
High	4	**61.1**	**0.001**	4	**16.8**	**0.015**	4	**94.7**	**0.001**	4	**21.8**	**0.010**	18	1.6	0.223	18	**10.6**	**0.004**
Medium	4	**21.8**	**0.010**	4	**13.5**	**0.021**	4	**9.5**	**0.037**	4	0.6	0.496	18	1.3	0.263	18	**5.8**	**0.027**
Low	4	**23.1**	**0.009**	4	3.3	0.142	4	1.0	0.383	4	0.0	0.882	18	1.5	0.238	18	0.0	0.886
Control 1	4	1.1	0.355	4	2.1	0.224	4	0.6	0.476	4	0.1	0.778	18	3.4	0.084	18	0.5	0.483
Control 2	4	**8.9**	**0.040**	4	0.7	0.451	4	**20.8**	**0.010**	4	0.1	0.738	18	0.9	0.359	18	0.4	0.550

Fig. 6. (A) Mean (±2 SE) percent 22:6n-3 (docosahexaenoic acid; DHA) and (B) mean (±2 SE) total 22:6n-3 (mg g^{-1} wet wt) in talitrid amphipods (*Traskorchestia* spp.) at 5 beach locations (High, Medium, Low, Control 1 & 2) in Quatsino Sound, British Columbia, in periods pre- and post-spawn of Pacific herring *Clupea pallasii* in March and April 2011. ANOVA significance between pairs: *p < 0.05 and **p < 0.01
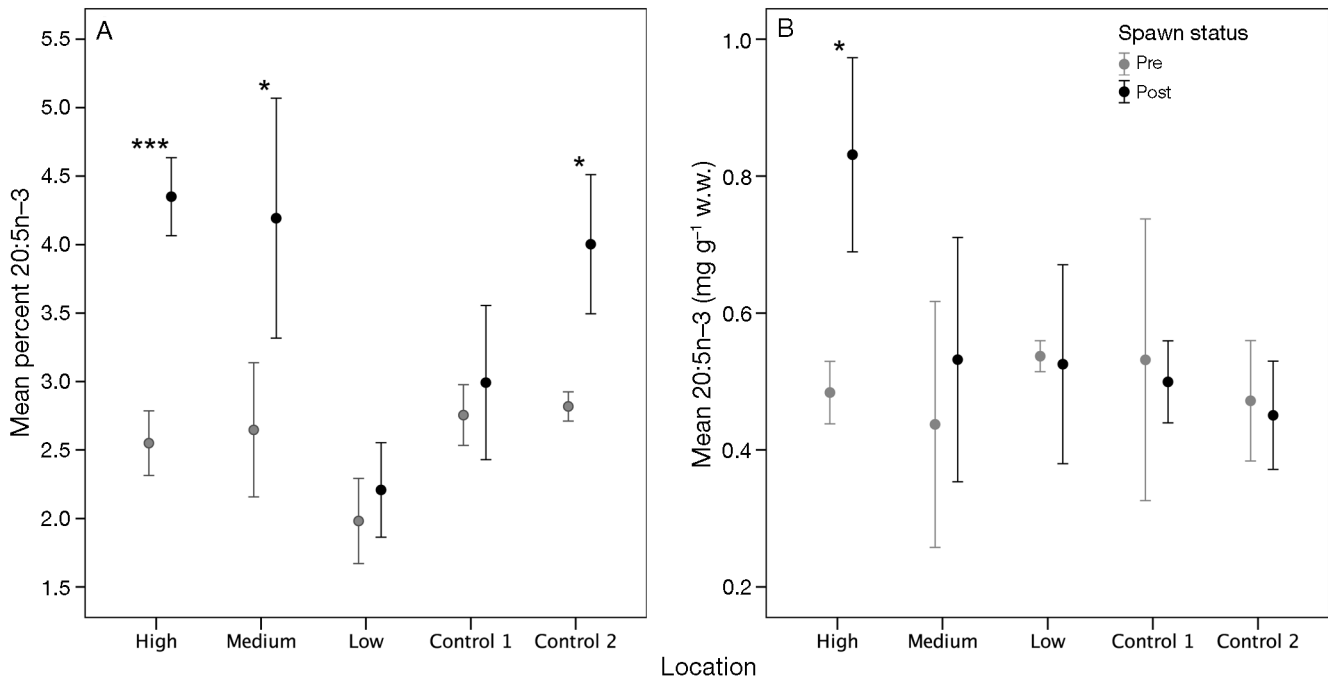

Fig. 7. Same as in Fig. 6 but for (A) mean (±2 SE) percent 20:5n-3 (eicosapentanoic acid; EPA) and (B) mean (±2 SE) total 20:5n-3 (mg g^{-1} wet wt) in talitrid amphipods (*Traskorchestia* spp.). *p < 0.05, **p < 0.01, ***p < 0.001

Stable isotopes

The presence of herring also significantly influenced $\delta^{13}C$ of amphipods (GLMM; df = 1, 49.6, F = 11.2, p = 0.002). Using 1-way ANOVAs, only amphipods from the High and Medium beaches demonstrated a significant decrease in their $\delta^{13}C$ signatures (High: p = 0.004, Medium: p = 0.027), and no significant changes were detected between before and after the spawn period on any other beaches for $\delta^{13}C$ (p > 0.05; Table 3, Fig. 8). The $\delta^{13}C$ of amphipods declined in the post-spawn period, becoming more similar to the depleted $\delta^{13}C$ signatures of Pacific herring eggs. In contrast, almost all macrophytes increased in $\delta^{13}C$ in the post-spawn period (Fig. 9). Although 1-way ANOVAs detected no significant differences on individual beaches before and after spawn periods (all beaches: p > 0.05; Table 3, Fig. 8), the general trend was of increasing $\delta^{15}N$ values, and a GLMM determined that cumulatively, the presence of herring had a significant influence on $\delta^{15}N$ values (df = 1, 52.2, F = 5.1, p = 0.028).

The use of MixSIR suggested that amphipods were heavily reliant on wrack subsidies and that eggs of Pacific herring were the dominant source of production on the High beach (estimated contribution of

Fig. 8. Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ isotopic values for talitrid amphipods (*Traskorchestia* spp.) on 5 beach locations: High (H, ◆), Medium (M, ◇), Low (L, ◆) and 2 Controls (C1, ○; C2, ●) in Quatsino Sound, British Columbia, in periods pre- and post-Pacific herring *Clupea pallasii* spawn event. Not displayed above, isotopic signatures for Pacific herring eggs lie at −18.3 ± 0.1 SE ($\delta^{13}C$) and 14.5 ± 0.1 ($\delta^{15}N$). *p < 0.05 in mean $\delta^{13}C$ values at 1 location before and after spawn. Dashed lines connect individual beaches in the pre- (0) and post-spawn (1) period

0.38 ± 0.12−0.58 CI), with smaller contributions to amphipods in Medium (0.07 ± 0.01− 0.25) and Low beaches (0.17 ± 0.02−0.35) (Fig. 9). Typically, *Phyllospadix serrulatus* and *Macrocystis integrifolia* were major sources of production on all beaches, and *Fucus* spp. contributed variably to amphipods across beaches and sampling periods, ranging from a minor contributor to a maximum of 0.26 ± 0.02−0.38 CI of the diet (Fig. 9). *Callithamnion* spp. and *Ulva lactuca*, when present, constituted minor wrack components and contributed little to the estimated diets of amphipods (Figs. 3 & 9).

DISCUSSION

Our study traces the allochthonous input of macrophyte wrack and Pacific herring eggs to semi-terrestrial talitrid amphipods. Living mainly in the high intertidal and supratidal zones, Talitrid amphipods are important beach detritivores (Koch 1990), agents of nutrient cycling and often highly abundant prey for terrestrial consumers (Orr et al. 2005). As likely consumers of both dead and live eggs, however, their feeding strategy in this study is better described as omnivorous. Although a number of previous studies have focused on the link between amphipods and macrophyte wrack (e.g. Talitrids; Lastra et al. 2008), the role of Pacific herring eggs as a source of production and a cross-ecosystem spatial subsidy to semi-terrestrial amphipods had never been previously explored.

Our broadest objective was to describe the major sources of production for *Traskorchestia* spp. Analysis of PUFAs of amphipods and their sources of production suggested that brown alga, represented here by wrack-dominant nearshore, canopy-forming *Macrocystis integrifolia* and abundant intertidal *Fucus* spp., were a major dietary resource. Stable isotope mixing models largely confirmed the importance of brown algae to the diet of amphipods and identified *M. integrifolia* as the major brown algal source. However, unlike PUFAs, stable isotope mixing models suggested that a major dietary component for amphipods is also the seagrass *Phyllospadix serrulatus*. Similar results have been found in an Australian surf zone amphipod (*Allorchestes compressa*), where stable isotope mixing models identified both brown algae and seagrasses as feasible carbon sources, and PUFAs suggested that brown algae was a dominant dietary component (Crawley et al. 2009). Previous research on wrack turnover and talitrid feeding rates have found that brown macroalgae is removed at
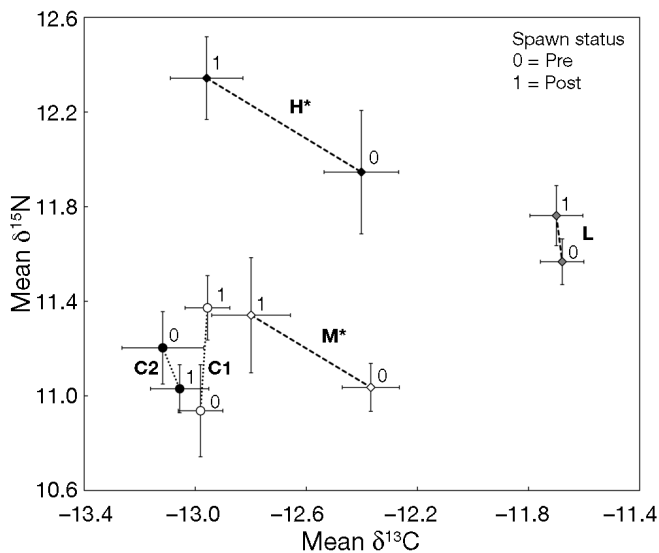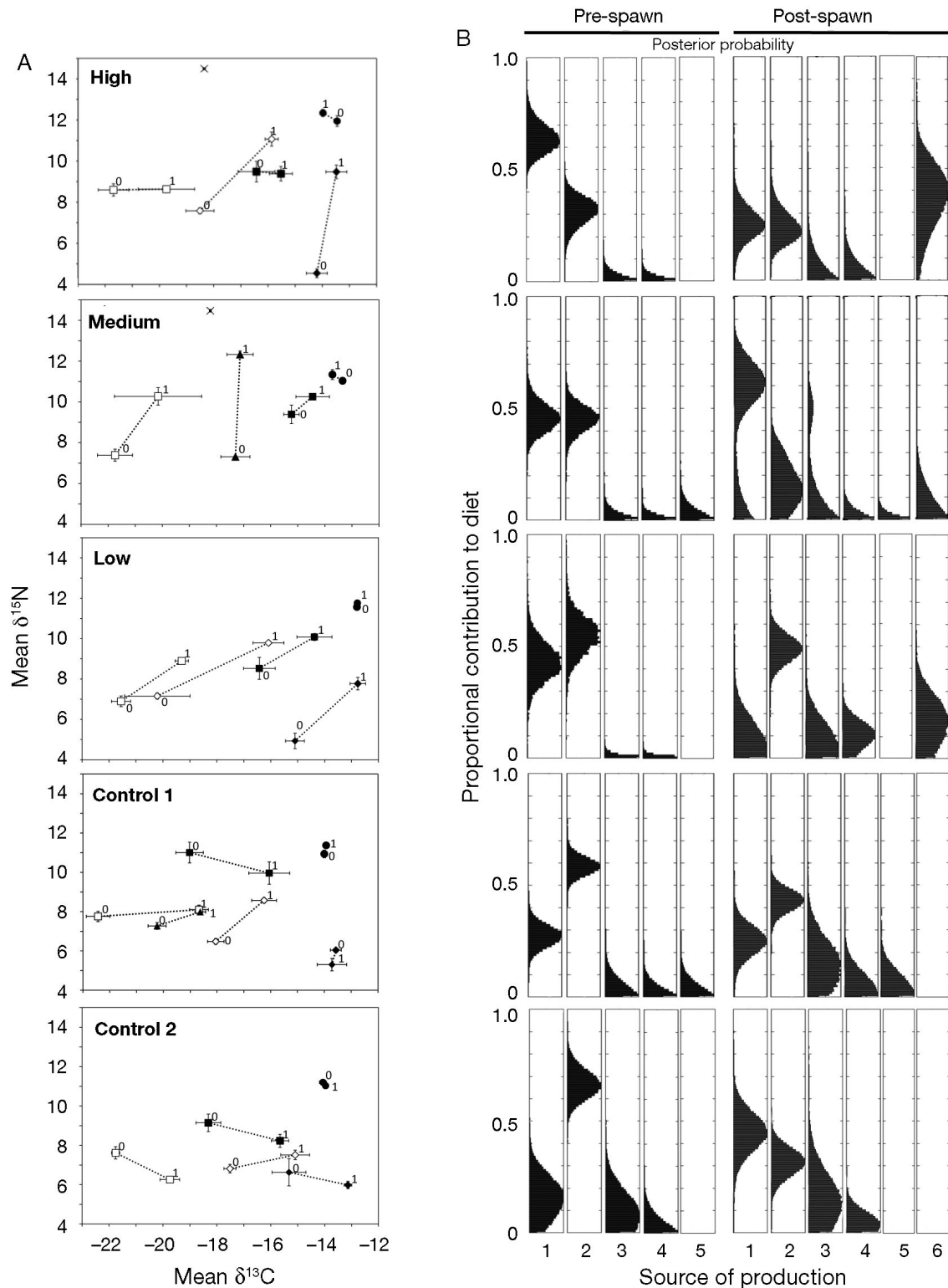
Fig. 9. (A) Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of potential sources of production for talitrid amphipods (*Traskorchestia* spp.) in 2011. (B) MixSIR estimates of the proportional contribution to the diet of talitrid amphipods by potential sources of production, including the eggs of *Clupea pallasii*, in periods pre- and post-spawn. For beaches where all sources of production could not be sampled (Low and Medium), stable isotopic averages were used from the 2 closest beaches (see 'Materials and methods'). Sources of production are *Phyllospadix serrulatus* (■, 1), *Macrocystis integrifolia* (◆, 2), *Fucus* spp. (◇, 3), *Callithamnion* spp. (□, 4), *Ulva lactuca* (▲, 5), eggs of *C. pallasii* (×, 6) and the detritivore consumers, *Traskorchestia* spp. (●). *U. lactuca* was not included as a potential source of production for High, Low and Control 2. *C. pallasii* was not included as a source of production for all pre-spawn beaches and post-spawn Controls 1 and 2

high rates but feeding is low to negligible on sea-grasses (Mews et al. 2006, Lastra et al. 2008), which corroborates our own observations (C. H. Fox) of the relatively rapid consumption of *M. integrifolia* by consumers, and the long 'wrack life' of *Phyllospadix* spp. Taken together, evidence suggests that *M. integrifolia* is a major resource for amphipods with contributions from *P. serrulatus*. Red and green algae, which are minor wrack components at our study sites, appear to provide negligible amounts to the diet of amphipods, as evidenced from both PUFA and stable isotope results. However, we note that our choice of enrichment factors in the stable isotope mixing models undoubtedly influenced the estimated contributions of macrophytes and herring eggs in diets of *Traskorchestia* spp.

Unlike wrack, which is available to intertidal and supratidal zone consumers on a fairly regular basis, the discrete nature of herring spawn events provide the opportunity to test the hypothesis that herring spawn resources provide a spatial subsidy to amphipods. GLMMs indicated that both δ^{13}C and δ^{15}N levels were significantly influenced by the presence of herring spawn. In more detail, significant differences in δ^{13}C on the post-spawn beaches that experienced high and medium egg loading, with a directional change for increasing similarity with the δ^{13}C signature of herring eggs, provided clear evidence for the uptake of herring-derived nutrients by amphipods. In addition to identifying *Macrocystis integrifolia* and *Phyllospadix serrulatus,* the eggs of Pacific herring were identified by mixing models as the dominant source of production on the high spawn beach, with smaller contributions of beaches with lower egg density.

Similar to stable isotope findings, NMDS ordination of amphipod PUFAs from all 5 beaches before and after the spawn revealed 2 significantly different groups, one consisting of amphipods from the High and Medium beaches in the post-spawn period, and the other group consisting of all remaining amphipods. Leading the PUFAs in contributing to these differences were DHA and EPA. EPA contributed the most to discriminating between the 2 amphipod clusters and although not as strong as DHA in supporting the hypothesis that herring spawn events subsidize amphipods, EPA provided corroborative evidence.

The presence of significantly elevated DHA, as measured using either relative proportions or raw concentrations, in amphipods on the post-spawn High and Medium beaches provided the strongest lipid-derived evidence of herring uptake. In the absence of herring, DHA proportions in amphipods

are generally <1%, and DHA is present at low to trace levels in the wrack species examined. Because DHA is present in low levels in red algae and amphipods without spawn, its elevated presence in post-spawn amphipods cannot be considered a lipid marker for Pacific herring. Instead, we suggest that in this system, DHA has the potential for use as an indicator FA, where concentrations are elevated in certain sources (Pacific herring) and low to negligible in others (Kelly & Scheibling 2012). In light of a recent feeding-trial study, where no consistent relationship between the FAs of an intertidal amphipod (*Bellorchestia quoyana*) and FAs from dietary macroalgae was found (McLeod et al. 2013), more research into this subject is warranted.

Beyond the use of stable isotopes, FAs and issues relating to a rapidly evolving field of study, additional questions arise that relate to the timing of the spawn, the size of the spawn and how these factors influence amphipods and the wider intertidal ecosystem. In addition, what are the consequences of nutritionally valuable PUFAs to amphipods and their predators (e.g. growth rates and demography), and how long do those effects last? Amphipods were sampled from beaches that experienced herring spawn the previous year, but no obvious stable isotopic or FA differences were observed in the spawn versus control beaches in the pre-spawn period, suggesting that this effect lasts less than a year. Post-spawn sampling occurred approximately 1 wk after egg hatch, meaning that this effect lasts at least 1 wk following hatch, a time when the spawn is traditionally considered 'over'. However, significant quantities of dead eggs and egg casings were observed to remain on beaches for several weeks following the hatch, and we speculate that the influence of herring spawn persists over time scales that range from several weeks to possibly months.

Given the life history of Pacific herring and knowledge of how lipids are obtained, the Pacific herring subsidy to amphipods can be traced in greater detail than provided by PUFAs and stable isotopes alone. In BC, most Pacific herring spawn from February to April, after which they migrate to summer foraging grounds in productive coastal waters (Hay et al. 2001), where they forage from late spring to early fall. During this time, they increase in length, mass and sequester important lipids (Hart et al. 1940), including DHA, EPA and other essential FAs (Huynh et al. 2007). Rich in DHA (Saito et al. 2002, El-Sabaawi et al. 2009), euphausiids and copepods are dominant prey items (Wailes 1936, Hay et al. 2001) and likely major sources of DHA for Pacific herring. In fall, herring move eastwards, towards more sheltered winter-

ing grounds (Hay et al. 2001), a migration that coincides with or, depending on timing, is followed by the onset of fasting/negligible feeding (e.g. Wailes 1936) and a declining growth rate (Outram & Station 1981). The following spring, the spawning of Pacific herring can thus be interpreted as the transfer of resources gained in productive offshore waters during the previous year to nearshore and intertidal zones at a time when primary production is low.

The federal management body for Pacific herring, Fisheries and Oceans Canada, considers Quatsino Sound Pacific herring a minor stock and limited monitoring in this region results in comparatively poor understanding of their population size and dynamics. However, commercial fishing has been closed for 3 of the 5 major meta-populations of BC Pacific herring due to low adult biomass, including the nearby West Coast Vancouver Island meta-population. In Quatsino Sound, it is highly likely that fewer fish return to spawn than they did historically. The reason(s) for widespread declines in BC Pacific herring populations and subsequent lack of recovery following 5 to 10 yr of fisheries closures remain largely speculative (see review by Schweigert et al. 2010), as are the ecological consequences of this declining cornerstone species (sensu Willson et al. 1998).

In particular, the ecological relationships between Pacific herring and intertidal and terrestrial ecosystems remain almost completely undescribed. In addition to tracing the macrophyte sources of production, this study represents the first evidence of a cross-ecosystem transfer of Pacific herring and links the lipids and nutrients gained by Pacific herring in seasonally productive coastal waters to semi-terrestrial invertebrates that inhabit intertidal and supratidal zones. Because amphipods are prey for a diversity of species, including terrestrial consumers, they represent a previously undocumented, indirect link between Pacific herring, intertidal and terrestrial ecosystems and may prolong access to herring spawn resources that are traditionally considered short-lived. Parallel to this study, research into the importance of amphipods in the springtime diets of inter-tidally-foraging black bears (*Ursus americanus*) has revealed that amphipods, including those inhabiting beaches that experience herring spawns, constitute a significant but annually variable food source for bears (Fox 2013). Currently, our understanding of coastal ecosystem interactions and consequences of anthropogenic pressures lack any meaningful Pacific herring subsidy context, despite substantial population declines in this important but overlooked species.

LITERATURE CITED

Alderdice DF, Hourston AS (1985) Factors influencing development and survival of Pacific herring (*Clupea harengus pallasii*) eggs and larvae to beginning of exogenous feeding. Can J Fish Aquat Sci 42:56–68

Alfaro AC, Thomas F, Sergent L, Duxbury M (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar Coast Shelf Sci 70:271–286

Álvarez-Romero JG, Pressey RL, Ban NC, Vance-Borland K, Willer C, Klein CJ, Gaines SD (2011) Integrated land–sea conservation planning: the missing links. Annu Rev Ecol Syst 42:381–409

Anderson EM, Lovvorn JR, Esler D, Boyd WS, Stick KC (2009) Using predator distributions, diet, and condition to evaluate seasonal foraging sites: sea ducks and herring spawn. Mar Ecol Prog Ser 386:287–302

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27: 325–349

Brett M, Muller Navarra D (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw Biol 38:483–499

Brower LP, Malcolm SB (1991) Animal migrations: endangered phenomena. Am Zool 31:265–276

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22: 759–801

Burke L, Kura Y, Kasem K, Revenga C, Spalding M, McAllister D (2001) Coastal ecosystems. World Resources Institute, Washington, DC

Colombini I, Chelazzi L, Gibson RN, Atkinson RJA (2003) Influence of marine allochthonous input on sandy beach communities. Oceanogr Mar Biol Annu Rev 41:115–159

Crawley KR, Hyndes GA, Vanderklift MA, Revill AT, Nichols PD (2009) Allochthonous brown algae are the primary food source for consumers in a temperate, coastal environment. Mar Ecol Prog Ser 376:33–44

Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Darimont CT, Paquet PC, Reimchen TE (2008) Spawning salmon disrupt trophic coupling between wolves and ungulate prey in coastal British Columbia. BMC Ecol 8:14

Darimont CT, Bryan HM, Carlson SM, Hocking MD and others (2010) Salmon for terrestrial protected areas. Conserv Lett 3:379–389

DeBlois EM, Leggett WC (1993) Impact of amphipod predation on the benthic eggs of marine fish: an analysis of *Calliopius laeviusculus* bioenergetic demands and predation on the eggs of a beach spawning osmeriid (*Mallotus villosus*). Mar Ecol Prog Ser 93:205–216

El-Sabaawi R, Dower JF, Kainz M, Mazumder A (2009) Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. Mar Biol 156:225–237

Ellis DW, Wilson S (1981) The knowledge and usage of marine invertebrates by the Skidegate Haida people of the Queen Charlotte Islands. Queen Charlotte Islands Mus Soc, Monogr Ser 1, Skidegate

Fisheries and Oceans Canada (2011) Section 273, Forward Inlet herring spawn records, catch and spawners. www. pac.dfo-mpo.gc.ca/science/species-especes/pelagic-pelagique/herring-hareng/herspawn/273fig-eng.html (accessed 9 Nov 2012)

Fox CH (2013) Novel interactions between black bears and Pacific herring. Pacific herring and salmon: ecological interactions across the land–sea interface. PhD dissertation, University of Victoria

Fry B (2006) Stable isotope ecology. Springer, New York, NY

Garman GC, Macko SA (1998) Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. J N Am Benthol Soc 17:277–285

Griffiths CL, Stenton-Dozey JME, Koop K (1983) Kelp wrack and the flow of energy through a sandy beach ecosystem. In: McLachlan A, Eramus T (eds) Sandy beaches as ecosystems. Junk Publishers, The Hague

Hart JL, Tester AL, Beall D, Tully JP (1940) Proximate analysis of British Columbia herring in relation to season and condition factor. J Fish Res Board Can 4:478–490

Hay DE, Toresen R, Stephenson R, Thompson M and others (2001) Taking stock: an inventory and review of world herring stocks in 2000. Herring: expectations for a new millennium. University of Alaska, Fairbanks, AK, p 381–454

Helfield JM, Naiman RJ (2001) Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology 82:2403–2409

Hocking MD, Reynolds JD (2011) Impacts of salmon on riparian plant diversity. Science 331:1609–1612

Huynh MD, Kitts DD, Hu C, Trites AW (2007) Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi.* Comp Biochem Physiol B 146:504–511

Kainz M, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol Oceanogr 49:1784–1793

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic foodwebs. Mar Ecol Prog Ser 446:1–22

Koch H (1990) Aspects of the population biology of *Traskorchestia traskiana* (Stimpson, 1857) (Amphipoda, Talitridae) in the Pacific Northwest, USA. Crustaceana 59: 35–52

Lastra M, Page HM, Dugan JE, Hubbard DM, Rodil IF (2008) Processing of allochthonous macrophyte subsidies by sandy beach consumers: estimates of feeding rates and impacts on food resources. Mar Biol 154:163–174

Lewis TL, Mews M, Jelinski DE, Zimmer M (2007) Detrital subsidy to the supratidal zone provides feeding habitat

for intertidal crabs. Estuar Coast Shelf Sci 30:451–458

Mancinelli G (2012) On the trophic ecology of Gammaridea (Crustacea: Amphipoda) in coastal waters: a European-scale analysis of stable isotopes data. Estuar Coast Shelf Sci 114:130–139

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390

McLeod RJ, Hyndes GA, Hurd CL, Frew RD (2013) Unexpected shifts in fatty acid composition in response to diet in a common littoral amphipod. Mar Ecol Prog Ser 479:1–12

Mews M, Zimmer M, Jelinski DE (2006) Species-specific decomposition rates of beach-cast wrack in Barkley Sound, British Columbia, Canada. Mar Ecol Prog Ser 328:155–160

Orr M, Zimmer M, Jelinski DE, Mews Z (2005) Wrack deposition on different beach types: spatial and temporal variation in the pattern of subsidy. Ecology 86:1496–1507

Outram DN, Station PB (1981) Assessing and monitoring maturity and gonad development in Pacific herring. Government of Canada, Fisheries and Oceans, Nanaimo

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainmann BC (eds) Lipids in freshwater ecosystems. Springer-Verlag, New York, NY

Polis GA, Hurd SD (1996) Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. Am Nat 147(3):396–423

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28: 289–316

Polis GA, Sánchez-Piñero F, Stapp PT, Anderson WB, Rose MD (2004) Trophic flows from water to land: marine input affects food webs of islands and coastal ecosystems worldwide. In: Polis GA, Power ME, Huxel GR (eds) Food webs at the landscape level. University of Chicago Press, Chicago, IL

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Reimchen TE (2000) Some ecological and evolutionary aspects of bear-salmon interactions in coastal British Columbia. Can J Zool 78:448–457

Saito H, Kotani Y, Keriko JM, Zue C and others (2002) High levels of n-3 polyunsaturated fatty acids in *Euphausia pacifica* and its role as a source of docosahexaenoic and icosapentaenoic acids for higher trophic levels. Mar Chem 78:9–28

Schoenecker KA, Singer FJ, Zeigenfuss LC, Binkley D, Menezes RS (2004) Effects of elk herbivory on vegetation and nitrogen processes. J Wildl Manag 68:837–849

Schweigert JF, Boldt JL, Flostrand L, Cleary JS (2010) A review of factors limiting recovery of Pacific herring stocks in Canada. ICES J Mar Sci 67:1903–1913

Semmens BX, Moore JW (2008) MixSIR: a Bayesian stable isotope mixing model, version 1.0. www. ecologybox.org (accessed 11 Aug 2012)

Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic feed web studies. Limnol Oceanogr 46: 2061–2066

Varpe Ø, Fiksen Ø, Slotte A (2005) Meta-ecosystems and biological energy transport from ocean to coast: the eco-

logical importance of herring migration. Oecologia 146: 443–451

Wailes GH (1936) Food of *Clupea pallasii* in southern British Columbia waters. Bull Biol Board Can 1:477–487

Walters AW, Barnes RT, Post DM (2009) Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. Can J Fish Aquat Sci 66:439–448

Wilcove DS, Wikelski M (2008) Going, going, gone: Is ani-

mal migration disappearing? PLoS Biol 6:e188

Willson MF, Womble JN (2006) Vertebrate exploitation of pulsed marine prey: a review and the example of spawning herring. Rev Fish Biol Fish 16:183–200

Willson MF, Gende SM, Marston BH (1998) Fishes and the forest. Bioscience 48:455–462

Womble JN, Sigler MF, Willson MF (2009) Linking seasonal distribution patterns with prey availability in a central place forager, the steller sea lion. J Biogeogr 36:439–451