The effects of live and artificial diets on feeding performance of cultured winter flounder, Pseudopleuronectes americanus, in the wild: survival, feeding, growth, and nucleic acid analyses

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Abstract

This research, which is part of a larger study designed to assess the feasibility of winter flounder, Pseudopleuronectes americanus, stock enhancement in New Hampshire, identifies hatchery feeds that optimize feeding-related performance of fish once released in the wild. Fish reared on post-nauplii of brine shrimp, Artemia sp.; white worms, Enchytraeus albidus; common burrower amphipods, Leptocheirus plumulosus; and formulated pellets were evaluated post-release from in-situ cages using survival, growth rate, feeding onset and incidence, stomach fullness, diet composition, and nucleic acid-based condition as indicators of hatchery-diet suitability. Amphipod-reared fish had the highest mean stomach contents index of all feed types, including wild fish. Wild and worm-reared fish exhibited the most similar survival, overall stomach fullness, and diet composition profiles over time. Amphipod-reared fish ranked highest in performance overall; however, if wild fish performance is viewed as the ideal for a stocked fish, worm-reared fish performed optimally. This study describes hatchery feeding strategies that may ease the transition of flatfish released into the wild for stock enhancement.

A major challenge of any captive rearing program is providing the appropriate feeding regimes during development. Typically marine fish larvae are initially fed live food (e.g. rotifers, Artemia), and then are weaned onto formulated diets as they attain a size or developmental state that supports consumption of these artificial feeds. Weaning onto formulated diets is a stressful time for cultured fish (Sterud et al. 2000), and this may be especially true for flatfish that have just undergone the dramatic morphological and physiological transformations associated with metamorphosis. Weaning occurs an additional time for stocked fish as they transition from formulated hatchery feed back onto live diets (i.e., wild diets) once released. Reared flatfish may take days (Sparrevohn et al. 2002; Fairchild 2010) to weeks (Ellis et al. 2002; Furuta et al. 1997) before they begin feeding consistently on wild prey and even then, non-conventional food items may be selected because of size/shape resemblances to formulated hatchery diets (Ellis and Nash 1998; Ellis et al. 2002). Selection of sub-optimal food items with high inorganic content, such as small stones or bivalves, may lower the physiological fitness of fish and affect survival (Howell 1973; Ellis and Nash 1998). This short period of starvation during the wild transition also can alter feeding behavior (e.g., increasing off-bottom swimming events), which may result in increased predation risk for reared flatfish (Furuta 1996; Miyazaki et al. 2000). Thus, conforming feeding performance of fish from the hatchery and the wild is paramount for any applied stock-enhancement effort.

Our objective was to quantify feeding-related performance of juvenile winter flounder, Pseudopleuronectes americanus, that were reared on different feeds (both live and formulated) in the hatchery (see Walsh et al., 2015). We evaluated how feeding history translated to the wild

feeding success once individuals were released into nature (caged in-situ) by examining survival, growth, feeding onset and incidence, stomach fullness, diet composition, and nucleic acid-based condition. Nucleic acid-based indices, such as the ratio RNA:DNA (RD), have been used to evaluate growth and nutritional condition of juvenile fishes since protein production varies in accordance with the quantity of RNA produced, while the DNA content of a cell remains relatively constant (Buckley 1980; Bulow 1987; Richard et al. 1991; Westerman and Holt 1994; Buckley et al. 1999). The RD ratio has been shown to respond to changes in feeding conditions and growth after short periods (1–3 d) in a variety of fish species, including larval and juvenile winter flounder (Buckley 1982; Buckley et al. 1999; Ben Khemis et al. 2000; Mercaldo-Allen et al. 2008).

Materials and Methods

Winter Flounder Rearing and Maintenance

From April–September 2008, winter flounder eggs, larvae and young juveniles were reared and maintained at the Coastal Marine Laboratory (CML), Judd Gregg Marine Research Complex, University of New Hampshire (UNH), in New Castle, New Hampshire, USA, as described by Walsh et al. (2015). In brief, after hatch larvae initially were fed a daily ration of rotifers, Brachionus plicatilis (enriched with DHA Selco; INVE, Salt Lake City, UT, USA). At 20 days after-hatch (DAH), in addition to rotifers, larvae were provided with enriched brine shrimp nauplii, Artemia sp. (enriched with DC DHA Selco; INVE). After 1.5 wk of co-feeding (30 DAH), rotifers were withdrawn and larvae were fed only brine shrimp nauplii through and

beyond settlement until initiation of weaning onto one of four different hatchery feeds (90 DAH): (1) a 0.5-0.8 mm mix of Skretting Gemma™ commercially-available, formulated pellets (Skretting USA, Tooele, Utah, USA); (2) brine shrimp post-nauplii; (3) white worms, Enchytraeus albidus; and (4) common burrower amphipods Leptocheirus plumulosus. Fish were weaned on their respective hatchery feed for a total of 14 d before trial initiation.

Wild Caging Trials

Surviving fish from the hatchery feeding trials of Walsh et al. (2015) were used in the wild caging trials in an eelgrass-surrounded, mud/silt-bottomed cove adjacent to the CML (43º 04' N; 70º 42' W; Fig. 1) from 03 September – 03 October 2008. During the 2-d transitional period between hatchery and cage trials, age-0 wild fish were seined from the cove, and all fish were tagged with color-coded visible implant elastomer tags (Northwest Marine Technology, Shaw Island, WA). Tag color distinguished wild or cultured individuals and feeding history of the cultured fish. Fish were not fed for 48 h prior to the initiation of the caging trials. This transitional, 2-d period also enabled fish time to recover from the handling stress of measurement and tagging (Sulikowski et al. 2005). Fish were released into 0.52 x 0.38 x 0.20 m cages (6 mm rigid metal square mesh; N=16) nested into larger, heavier cages (0.9 x 0.6 x 0.45 m; 11 mm rigid metal square mesh) to weigh down and stabilize them in the mud, as well as to provide an additional barrier against predators. Three fish of each feed type plus three wild fish (i.e., fifteen fish total) were released per cage so that performance means with standard errors could be calculated for each treatment. Wild fish (58.48 ± 1.22 mm standard length, SL) seined from the

cove and worm-reared fish (38.80 ± 0.52 mm SL) both were statistically larger than brine- (34.82 ± 0.51 mm SL), amphipod- (32.28 ± 0.55 mm SL), and pellet-reared (32.26 ± 0.69 mm SL) fish at the start of cage trials (Fig. 2); however, all hatchery-reared fish were from the same cohort with size differences resulting from the provision of different hatchery feeds (Walsh et al., 2015). All fish were not released at the same time; rather, releases were scattered over a 27-h period (3-4 September 2008) so that all retrievals occurred during daylight hours (Fig. 1). Cages were deployed at least 2 m from one another and were retrieved between 3 h and 30 d post release, resulting in a gradient of 16 distinct cage-hauling events (Table 1). Retrieval timeframes were chosen to establish a matrix of continuous performance data up to one month post-release, particularly for detecting onset of feeding and changes in nucleic acid condition. Due to mortality during hatchery trials and the subsequent 2-d transitional period, all hatchery feed types were not represented in all cages during wild trials. Fish were snap-frozen on dry ice upon retrieval. Subsequently, fish were measured (SL) in the hatchery, and digestive tracts were removed and preserved in formalin for stomach content analyses.

Weight measures were not possible immediately upon cage retrieval due to the constraints of working in the field, and once snap-frozen, additional water weight accompanying such small specimens made weight measures highly inaccurate. Therefore, growth data are presented in terms of SL only. Instantaneous growth rates (G_{SL}/d) and somatic growth rates (mm/d) were calculated (Table 2); values for fish retrieved > 3 d post cage release were emphasized to ensure that growth estimates reflected wild feeding activity. Mean growth rates per feed type per cage were compared by 1-way ANOVA, followed by Tukey-Kramer Multiple

Comparisons Test. The influence of hatchery feed type on survival upon cage retrieval was assessed via Chi-square association. To aid visualization of diet composition and stomach fullness over time, data were compiled into four time groups post cage release: within 1 d (0.125, 0.25, 0.5 and 1 d); from 1 d to 1 wk (1.25, 1.5, 2, 3, and 5 d); approximately 2 wk (9, 12, 16 d); and approximately 1 mo (19, 23, 26, 30 d). These groupings were chosen to examine the extent to which hatchery feeds led to immediate feeding onset in the wild, as well as to determine what those immediate feeders chose to eat (within 1 d); feeding onset of those fish that, according to Fairchild (2010), may take 3-4 days before feeding on wild prey (from 1 d to 1 wk); food choice of all fish – all should be feeding by this time (approximately 2 wk); and food choice over the longer term, as wild prey availability within cages becomes limiting over time (Fairchild et. al, 2005), which may lead to increased competition between individuals (approximately 1 mo).

The Index of Relative Importance (IRI; Pinkas et al. 1971) was applied to describe prey composition of the stomach contents of the cage-released fish. Stomach fullness was indicated by the Stomach Contents Index (SCI). SCI within (over time, by time group) and between (overall) hatchery feed types was compared by Kruskal-Wallis followed by Dunn's Multiple Comparison Test since data did not conform to a Gaussian distribution.

Water quality data were obtained from a DataSonde buoy deployed at the end of the UNH pier (Fig. 1) as part of the Great Bay National Estuarine Research Reserve System Wide Monitoring Program and the UNH DataSonde Program. The DataSonde recorded measurements of water temperature, salinity, dissolved oxygen, pH, and turbidity at approximately 30-min

intervals; we used temperature and salinity data collected nearest to 12:00 PM for the duration of caging trials.

Nucleic Acid Analyses

In preparation for analyses, frozen fish were dissected on a tray set on ice. White muscle tissue samples consisted of the fillet from the dorsal side. Dissecting tools were rinsed with deionized water between dissections to avoid contamination. Each tissue sample was weighed to the nearest 0.001 g and placed in a test tube in an ice slurry bath. The tissue was homogenized in ice-cold distilled water using a Janke and Kunkel Ultra-Turrax tissue homogenizer. Replicate aliquots immediately were frozen and biochemical analyses of the tissues were completed within 48 h of freezing.

Muscle tissue samples were analyzed using a UV-based method according to Buckley and Bulow (1987) as modified by Kuropat et al. (2002). First, free nucleotides were removed using a series of washes with cold perchloric acid ($HClO_4$). RNA was then hydrolyzed with potassium hydroxide and the hydrolysate was acidified with cold $HClO_4$ to remove the RNA from the DNA and protein. Then DNA was both hydrolyzed and separated from the remaining protein by the addition of hot $HClO_4$. RNA and DNA were estimated from the absorbance of the appropriate hydrolysate at 260 nm using the following extinction coefficient: A_{260} of a 1-μ g/ml solution of hydrolyzed RNA or DNA is 0.03. Absorbance was measured using a Ciba-Corning Gilford Response Spectrophotometer. RNA and DNA concentrations were calculated as μ g/mg wet tissue weight. As a quality control measure, a large quantity of scup, Stenotomus chrysops,

muscle tissue was homogenized and frozen in 0.2 g aliquots. One control sample was processed each day along with the tissue samples to verify the accuracy of the run (Buckley et al. 1999).

RNA quantities reflect growth 1–3 d prior to sampling (Kuropat et. al 2002), and fish were not fed for 2 d before cage release; therefore, RD ratio and RNA concentrations of fish retrieved ≤ 1 d post cage release were considered baseline values reflective of hatchery feeds. Baseline values of RNA and DNA concentration among hatchery feeds were compared via ANOVA, followed by Tukey's Multiple Comparisons test, while those of RD ratio were examined via Kruskal-Wallis, followed by Dunn's Multiple Comparisons test because the variances among hatchery feeds were unequal. Juvenile winter flounder may take 3–4 d before they begin feeding on live prey once released (Fairchild 2010), so only values for fish released > 3 d (5–30 d post release) were considered indicative of wild-weaned fish to ensure that growth estimates were due to wild-feeding activity. Spearman correlations were conducted to test the relation between instantaneous growth rate, RD ratio, RNA and DNA concentrations, temperature, and time post release for all fish retrieved > 3 d. RD ratio, and RNA and DNA concentrations also were plotted by day of cage retrieval for fish retrieved > 3 d, and values within feed types were examined by linear regression to describe general trends over time.

Results

Wild Caging Trials

For the duration of wild cage release trials, seawater temperatures ranged from 11.4 to 19.0 ºC (mean 15.2 ± 0.4 ºC) and salinities from 25.7 – 31.4 ppt (mean 29.3 ± 0.3 ppt). There

were no significant associations between fish raised on different feeds and survival ($\chi^2 = 8.56$, $P = 0.07$). Wild, worm-, and amphipod-reared fish all exhibited over 90% survival during caging (Fig. 3).

The majority of empty guts (stomach + intestines) were observed within the first 6 h after release for all hatchery feed types (data on pellet-reared fish not available; Fig. 4). After 1 d post release, wild fish had the highest incidence of empty guts. Overall mean SCI was highest for amphipod-reared fish (1.32) and was lowest for wild fish (0.36; KW = 23.32, $P < 0.001$, Fig. 5a). Over the course of the caging period, worm- and brine shrimp-reared fish showed significant increases in SCI from the first day post release (KW = 12.15, $P < 0.01$ and KW = 17.83, $P < 0.001$, respectively; Fig. 5b). Overall diet composition was similar between fish reared on all hatchery feed types and wild fish. Identifiable prey found in the stomachs of cage released fish included polychaetes, amphipods, copepods, bivalves, cumaceans, nematodes, decapods, isopods, arthropods, gastropods, and tunicates with only the first five prey categories making up the bulk of dietary importance as per IRI (Fig. 6).

Length-based growth rates for individual fish retrieved > 3 d post cage release (reflecting growth based on the wild-transition diet) ranged from 0.0001 to 0.0242 G_{SL}/d (instantaneous) and from 0.0087 to 1.01 mm SL/d (somatic). Growth rates of worm- and brine shrimp-reared fish were similar while in the cages (Table 3). Overall, wild fish had significantly lower instantaneous growth rates than all hatchery-reared fish (F = 14.56, $P < 0.0001$), which corresponds with their larger body size at release (Fig. 2).

Nucleic Acid Analyses

Fish retrieved > 3 d post cage release showed a significant positive correlation between length-based instantaneous growth rate and RD ratio, RNA concentration, and DNA concentration (Table 4). RNA concentration showed the strongest association with length-based growth. DNA concentration and G_{SL} were negatively correlated with days post release (time) and positively correlated with seawater temperature. Water temperature and time post release were not correlated with RD ratio. There were no significant differences in RD ratio (KW = 6.39, P = 0.17), RNA concentration (F = 1.37, P = 0.26), or DNA concentration (F = 1.92, P = 0.12) between baseline values for any feed type. Once released, amphipod-reared fish exhibited the highest RD ratio, although not significantly different from pellet- or brine-reared fish (F = 4.38, $P < 0.01$; Fig. 7a), and pellet-reared fish had significantly higher RNA (F = 33.75, $P < 0.0001$; Fig. 7b) and DNA concentrations (F = 7.19, $P < 0.0001$; Fig. 7c). Over time, RD ratio did not significantly change over time for any hatchery feed type (Fig. 7d). RNA concentration significantly decreased over time for pellet-reared fish ($r^2 = 0.51$, $P < 0.05$; Fig. 7e). There were no significant changes in DNA concentration over time while fish were in the cages, although DNA tended to decrease for all feed types (Fig. 7f). Overall, wild fish maintained the most stable RD and RNA and DNA concentrations over time (Fig. 7d, e, f).

Discussion

Wild Caging Trials

Most cage mortality occurred during the last 2 wk of trials. There were 15 fish in each cage, and as fish grew over the course of the trials, it is likely that food availability in the cages became limited over time. Fairchild et al. (2005), who examined caged juvenile winter flounder from the same estuary as the present study, found that wild prey availability (i.e., polychaetes, amphipods, nematodes, bivalves, and cumaceans) inside of release cages decreased over a 10-wk period compared to that outside of cages. Although the percentage of empty stomachs did not increase and SCI remained constant or increased with time, fish were growing and thus gaining a higher food demand in a limited feeding environment. This high density of fish in a small space may mimic the high-density, point source release of a true stocking effort. Food resources become available when lesser competitors die, thus resulting in increased stomach fullness for some feed types in conjunction with the lower survival of others over time. Note, however, that all survival trends expressed here represent that of fish in a predator-free environment, and we would expect even higher mortality in the presence of predators. How type of hatchery-feed may influence avoidance behavior and survival in the presence of predators is still unknown.

Ellis and Nash (1998) and Ellis et al. (2002) suggested that the occurrence of some sub-optimal "prey" items, such as small stones, in the stomachs of recovered hatchery-reared fish may be due to the resemblance of these items to formulated pellet feeds. In the present study, we did not detect stones in the stomachs of fish, and although there was a high incidence of similar-shaped bivalves in the stomachs of fish, the amount observed in pellet-reared fish did not differ from fish reared on any other hatchery feed (note that other hatchery feed types were never exposed to formulated feeds at any point in their lifetime). The incidence of bivalves in the

stomachs of all fish types, including wild fish (although wild fish consumed the least amount overall), increased as fish approached 2 wk post release.

Growth rates of worm-reared fish were slightly lower in the cages (0.008 G_{SL}/d; 0.31 mm/d) compared to growth performance in the hatchery (0.010 G_{SL}/d; 0.37 mm/d; Walsh et al., 2015). However, brine shrimp-, amphipod- and pellet-reared fish all exhibited higher growth rates while in the cages. Somatic growth rates of fish caged > 3 d in the present study correspond to those at the same locale for hatchery-reared juvenile winter flounder reported in earlier years (Fairchild 1998; Fairchild et al. 2005). These growth rates also overlap the lower range of values for wild juvenile winter flounder reported by previous caging studies conducted at much lower densities in warmer, more southern locations (Table 5).

Nucleic Acid Analyses

The significant positive correlations between nucleic acid indices and length-based instantaneous growth rate in our study are evidence that RNA concentration and RD ratio represent good proxies for growth rate in juvenile winter flounder. It is well established that RNA and RD are good indicators of growth in winter flounder (Buckley 1982; de Montgolfier et al. 2005; Kuropat et al. 2002) and generally (Buckley 1980; Richard et al. 1991; Clemmesen 1994; Westerman and Holt 1994; Ben Khemis et al. 2000; Gwak and Tanaka 2001). The lowest value detected for an individual in the present study was 1.82 (one brine shrimp-reared fish) and only five fish had a value below 2 (one wild, one worm-, and three brine shrimp-reared fish). Thus, most fish were able to maintain themselves above starvation level while in cages.

A number of studies have examined RNA and DNA concentrations of young winter flounder at various developmental and nutritional states (Buckley 1980; Ben Khemis et al. 2000; de Montgolfier et al. 2005; Mercaldo-Allen 2008; Fraboulet et al. 2010) and habitats (Kuropat et al. 2002). The mode in the frequency distribution of RD values in the present study fell between 3 and 3.5, the same as that recorded by Mercaldo-Allen et al. (2008) when examining wild-collected juvenile winter flounder held in the laboratory at similar temperatures. de Montgolfier et al. (2005) and Fraboulet et al. (2010) both recorded lower mean values (< 3) for juvenile winter flounder from colder, Canadian waters.

RD ratio of juvenile Japanese flounder, Paralichthys olivaceus, fed live mysids for 8 d was 1.7 times higher than those of juveniles fed artificial feeds (Gwak et al. 2003). We saw no evidence of this trend in the present study when examining baseline RD values reflective of wild, live, and formulated feeds. Although nucleic acid data for pellet-reared fish were limited due to lower survival rates, the present study indicates that fish reared on formulated feeds are able to maintain an RD ratio comparable to fish fed live feeds in the hatchery even when growth rates are much lower, and are able to maintain that RD ratio after transitioning to natural diets in the wild; however, the impact of a pellet-reared diet on survival must be considered.

Implications for Stock Enhancement

Pellet-reared fish had 72% total mortality from the initiation of all experiments: 36% from 28-d hatchery feeding trials (Walsh et al., 2015); 28% from the 2-d transitional period between hatchery and wild trials; and 8% of total mortality from 30-d wild caging trials.

Therefore, although pellet-reared fish released in wild cages exhibited similar feeding, growth, and nucleic acid values to those of other hatchery feed types, the impact of lower survival overall cannot be overlooked in the context of a stocking effort. The present study only considers post-release, non-predation induced mortality. We expect that mortality would increase in the presence of predators. In addition, we did not consider any post-release behavioral benefit to rearing fish on live hatchery feeds.

Cage-released brine shrimp- and amphipod-reared fish had higher mean SCI and RD ratio among all feed types, indicating these fish were hunting more actively. By being reared on highly motile live feeds that swim in 3-dimensions, these fish may have gained better training for the life of an active predator than those fish reared on other feeds. However, the active foraging exhibited by brine shrimp- and amphipod-reared fish may be sub-optimal to survival with prolonged exposure to predators in the wild. Burke and Masuda (2010) suggested that bold feeding behaviors developed in the hatchery may be a poor strategy for flatfish, which need to be both stealthy predators as well as cryptic prey once released.

If we are to consider wild fish as the norm, then the overall performance of worm-reared fish most consistently matched that of wild performance. Wild and worm-reared fish exhibited the most similar survival, overall SCI, and diet composition profiles over time. This is not surprising, as worms (polychaetes) were the major component of the wild flounder diet overall, and polychaetes are a major natural prey component of winter flounder, including those < 1 yr old (Pearcy 1962; Festa 1979; Stehlik and Meise 2000). However, because of the 2-cm size difference, some caution should be taken in directly comparing performance between wild and

hatchery-reared fish in this study. In addition, it should be noted that wild fish are the survivors of a natural selection process in the field that includes utilizing natural prey and avoiding predators.

Conclusions

Cage-release trials indicated that amphipod-reared fish maintained the highest SCI and RD ratios, and there were no statistical associations between survival and hatchery feed type. A ranked summary of all hatchery feed types in both hatchery (Walsh et al., 2015) and cage trials reveals that amphipod-reared fish ranked highest in performance overall (Table 6). However, worm-reared fish exhibited the highest hatchery performance, and in caging trials worm-reared and wild fish exhibited the most similar survival, overall SCI, and diet composition profiles over time. Therefore, if we designate the performance of wild fish as the ideal for a stocked fish, worm-reared fish were the optimal performers. We should also note that overall performance could only be assessed for survivors. Thus, examining survival in this rank summary is a prerequisite before considering any other additional performance measure. In addition, how performance would be influenced by the presence of predators is still unknown.

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TABLE 1. Cage retrieval schedule for fish reared on different hatchery feeds. Letters denote analyses conducted on fish from specific cages: "a" = survival, "b" = growth, "c" feeding (onset, stomach fullness, diet composition), "d" = baseline RNA/DNA (ratio, composition), "e" = wild cage feeding RNA/DNA (ratio, composition). Each sampling consists of termination of cage and complete sampling of fish.

	Days post-release															
Hatchery feed	0.125	0.25	0.5	1	1.25	1.5	2	3	5	9	12	16	19	23	26	30
Worm	abcd	abcd	abcd	abcd	abc	abc	abc	abc	abce	abce	abce	abce	abce	abce	abce	abce
Brine shrimp	abcd	abcd	abcd	abcd	abc	abc	abc	abc	abce	abce	abce	abce	abce	abce	abce	
Amphipod	abcd	abcd	abcd	abcd	abc	abc	abc	abc	abce	abce	abce	abce		abce		abce
Pellet			abcd	abcd	abc	abc	abc	abc	abce	abce		abce				abce
Wild	abcd	abcd	abcd	abcd	abc	abc	abc	abc	abce	abce	abce	abce	abce	abce	abce	abce

TABLE 2. Statistics calculated to describe feeding performance.

Statistic	Formula	Variables
Somatic growth rate	$= (Y_z - Y_i)/T$	Y_i = length or weight at initial time Y_z = length or weight at time z T = time period
Instantaneous growth rate (G)	$= \ln(Y_z/Y_i)/T$	Y_i = length or weight at initial time Y_z = length or weight at time z T = time period
Index of relative importance (IRI)	$= (N+V)(F)$	N = % numerical composition of prey category within the individual V = % dry weight of prey category within the individual F = % frequency of occurrence of prey category within the sample population
Stomach contents index (SCI)	$= (W_{sc} * 100)/(W_f - W_{sc})$	W_{sc} = stomach content dry weight W_f = fish total body dry weight

TABLE 3. Mean growth rates (± SEM) of winter flounder retrieved from cage experiments > 3 d post-release. Letters indicate significant differences between hatchery feed types within each growth measure ($P < 0.05$).

Feed type	Instantaneous growth rate (GSL/d)	Somatic growth rate (mm/d)
Worm-reared	0.0076 ± 0.0007[a]	0.31 ± 0.03 [ab]
Brine shrimp-reared	0.0079 ± 0.0009 [a]	0.30 ± 0.04 [ab]
Amphipod-reared	0.0122 ± 0.0012 [ab]	0.44 ± 0.05 [a]
Pellet-reared	0.0136 ± 0.0030 [b]	0.47 ± 0.11 [a]
Wild	0.0025 ± 0.0005 [c]	0.15 ± 0.04 [b]

TABLE 4. Correlation coefficients of biochemical parameters, growth, time, and seawater temperature for fish retrieved > 3 d post release. Correlations were run with means of all retrieved fish per feed type per cage. *, **, ***, and **** indicate significance at $P \leq 0.05$, 0.01, 0.001, and 0.0001, respectively; ns = not significant. Correlations between RNA/DNA ratio and RNA and DNA concentration, respectively, were not calculated due to the confounding nature of these measures.

	RNA/DNA	RNA	DNA	Instantaneous growth rate	Time
Temperature (C)	-0.19 ns	0.18 ns	0.36 *	0.34 *[1]	-1 ****
RNA/DNA (ratio)				0.54 ***[2]	0.19 ns
RNA (µg/mg wet tissue Wt)			0.62 ****	0.81 ****	-0.18 ns
DNA (µg/mg wet tissue Wt)				0.40 *	-0.36 *
Instantaneous growth rate (G_{SL} d^{-1})					-0.34 *[1]

[1] $P = 0.05$

[2] $P = 0.001$

TABLE 5. Juvenile winter flounder growth rates reported from previous wild caging studies conducted in-situ. All locations are in northeastern USA. SL = standard length; TL = total length; W = wild fish; HR = hatchery-reared fish; N/A = not available.

Release location	Release size (mm)	Release duration	Water temperature (C)	Recovery (%)	Somatic growth rate (mm/d)	Cage size (m; length x width x height)	Cage density (# of fish /cage)	Fish type (W or HR)	Reference
New Jersey	22–84 TL	10 d	19–27	23-97	-0.15 to 1.30	1 x 1 x 0.46	3	W	Sogard, 1992
New Jersey; New York	14–29 SL	11 d	11–26	61-94	-0.06 to 0.53	0.85 x 0.85 x 0.45	3	W	Able et al., 1999
New Jersey; Connecticut	16–46 SL	9–11 d	16–35	17-91	-0.03 to 0.69	0.72 x 0.72 x 0.45	3	W	Phelan et al., 2000
New Jersey	18–38 SL	10 d	10–28	56-100	0.23 to 0.56	0.85 x 0.85 x 0.45	3	W	Curran and Able, 2002
New Jersey	20–24 SL	12 d	13–29	73-89[a]	0.00 to 0.90	0.75 x 0.75 x 0.40	3	W	Manderson et al., 2002
Rhode Island	33–37 TL	10–15 d	14–27	N/A	0.29 to 0.44	1 x 1 x 0.70	4	W	Meng et al., 2000
Rhode Island	30–37 TL	15 d	18–21	94[a]	0.22 to 0.60	1 x 1 x 0.70	4	W	Meng et al., 2001
Rhode Island	25–35 TL	14–16 d	14-27	89	0.51 to 0.95	1 x 1 x 0.70	4	W	Meng et al., 2008
New Hampshire	40-58 TL (W) 32-46 TL (HR)	10 wks	7-22	22 (W) 50 (HR)	0.06	1 x 1 x 1	20	W, HR	Fairchild, 1998
New Hampshire	36 TL	7 wk	11-27	47-56	0.37 to 0.56	1 x 1 x 1	5	HR	Fairchild et al., 2005

[a]Does not include fish resulting from non-recovered cages.

TABLE 6. Performance rank summary of fish reared on all hatchery feed types in both hatchery and wild cage trials. The number 4 denotes the highest rank and 1 the lowest rank of all feed types. Fractional ranking was employed in cases where more than one hatchery feeding type occupied the same rank seat. With the exception of survival, all performance measures were ranked based on the mean value over the course of trial. Growth measures were based on instantaneous growth in standard length.

		Worm-reared	Brine shrimp-reared	Amphipod-reared	Pellet-reared
Hatchery	Survival[a]	3	3	3	1
	Growth	4	3	2	1
Wild cage	Survival[a]	3.5	2	3.5	1
	Growth[b]	1.5	1.5	3	4
	Feeding incidence[c]	2	1	3	4
	Stomach fullness	1	2	4	3
	RNA/DNA ratio	1	2	4	3
Totals	Hatchery	7	6	5	2
	Wild Cage	9	8.5	17.5	15
	Overall	16	14.5	22.5	17

[a] All survival over 90% was given highest rank.

[b] Growth measures for worm- and brine shrimp-reared fish were given equal rank since their values equaled to the 0.001 level.

[c] The percent of empty guts (where highest number denotes lowest performance) was converted to its inverse, percent feeding incidence (where highest number denotes highest performance) for ranking purposes.

FIGURE 1. Arrangement of cage distribution for fish released in the cove adjacent to the Coastal Marine Laboratory (CML) of the University of New Hampshire. Each small rectangle represents one cage. Number inside rectangle denotes day of retrieval after release. Number below rectangle indicates the time of day individual cage was retrieved. Large bold square represents floating dock from which cages were deployed. Figure not to scale.

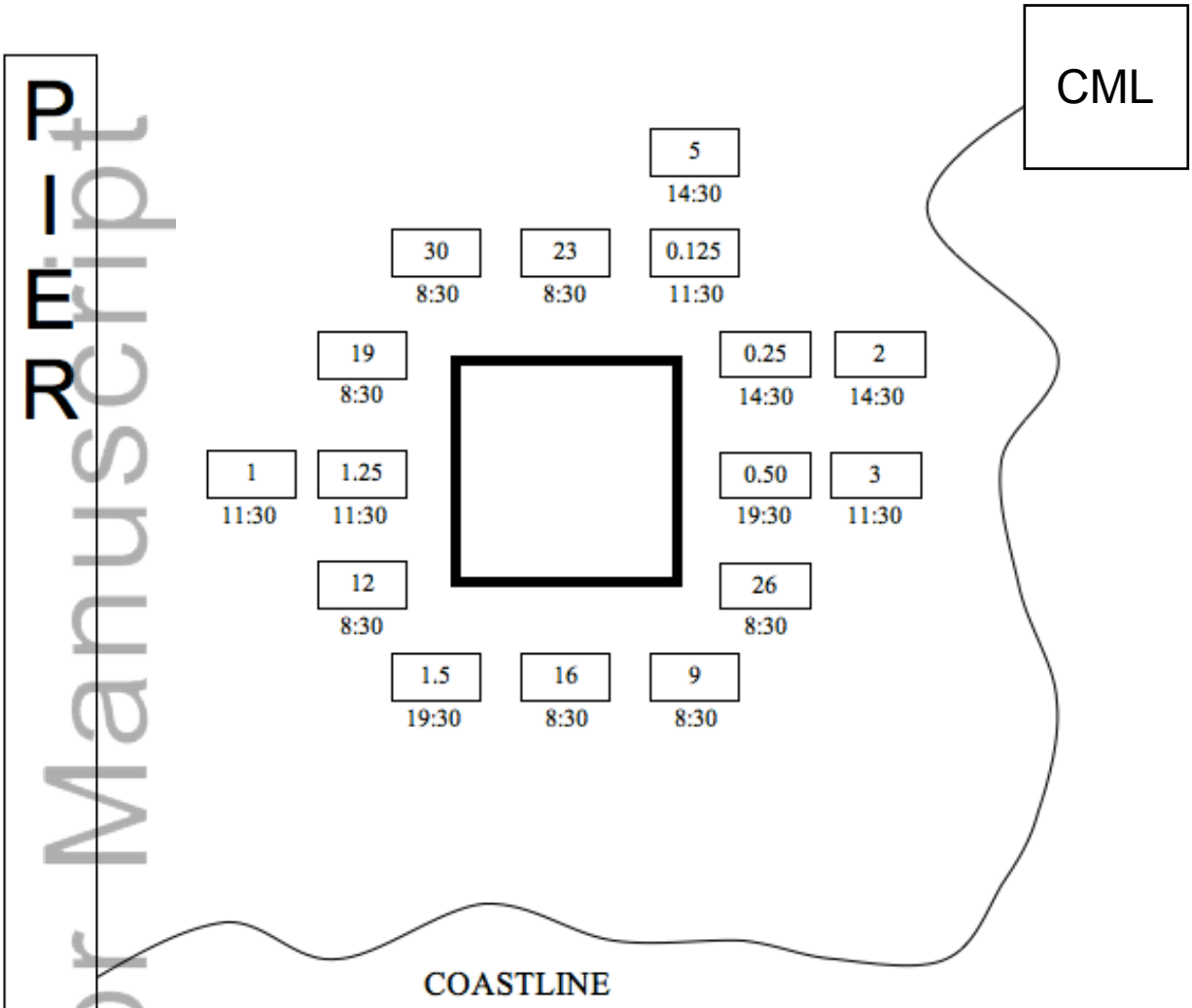

FIGURE 2. Sizes of fish released into cages. Letters above box plots indicate significant differences in standard length between feed types ($P < 0.05$).

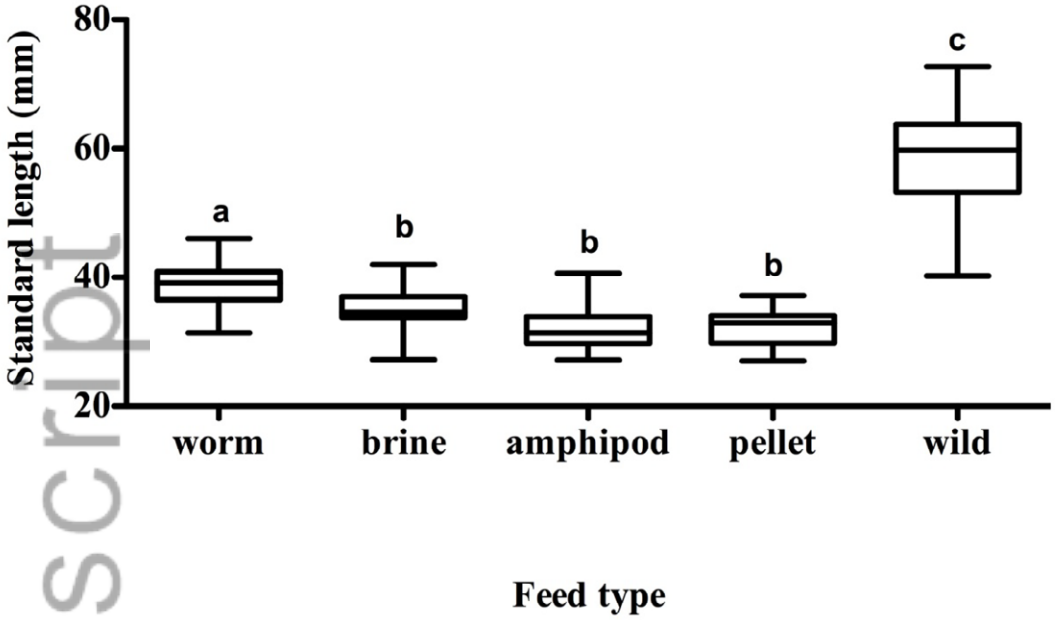

FIGURE 3. Cumulative survival of caged fish reared on different feeds in the hatchery expressed as percentage over time. There was no association between hatchery feed type and survival upon cage retrieval.

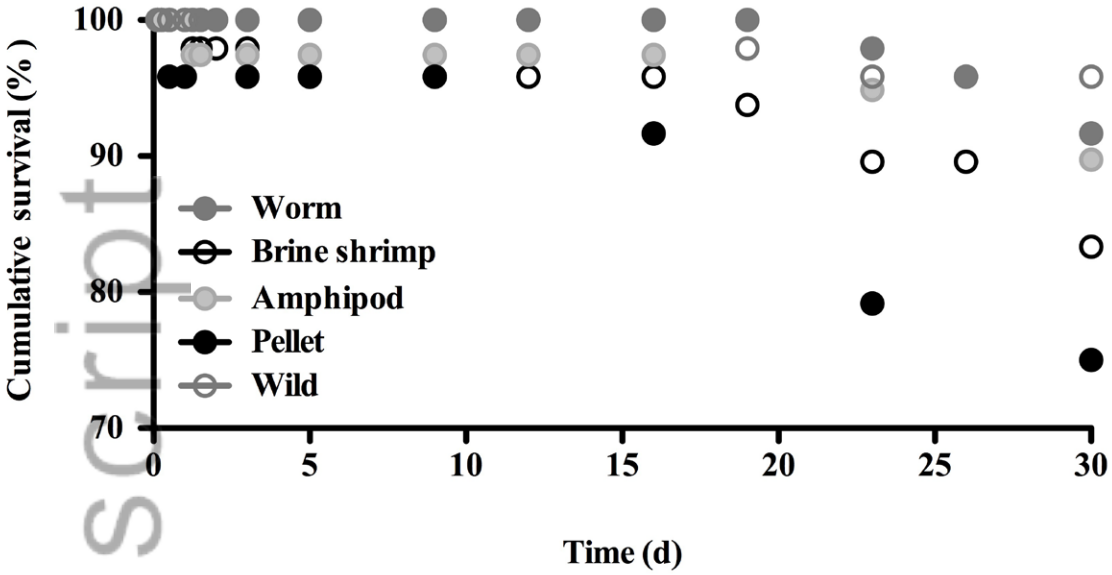

FIGURE 4. Percent empty guts (stomach + intestines) at days post release. Underlines on x-axis values indicate time intervals when no information for pellet-reared fish is available; double underlines on x-axis values indicate time intervals when no information for pellet- or amphipod-reared fish is available. No empty guts were detected after 19 d.

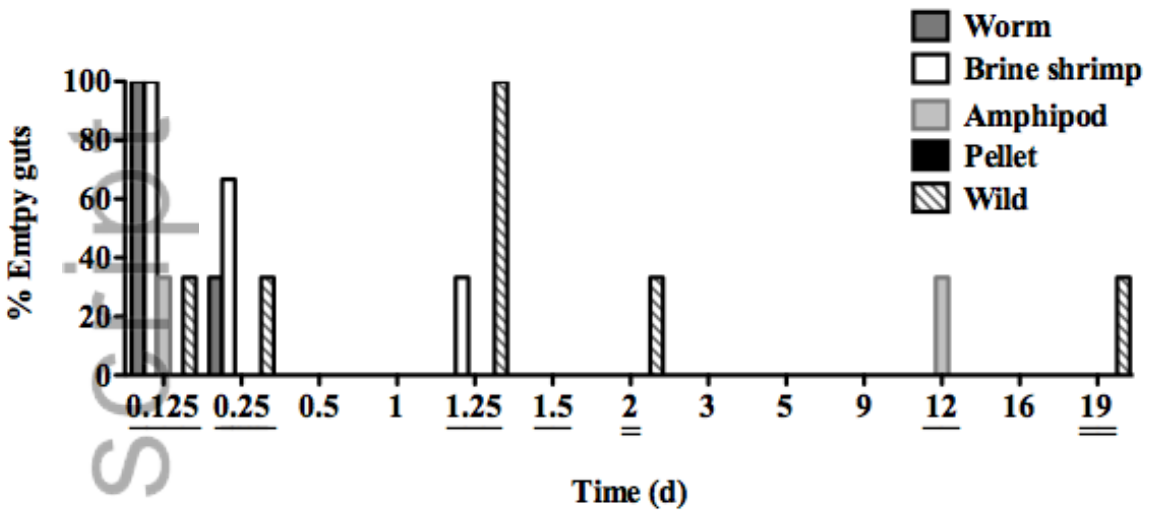

FIGURE 5. Mean Stomach Contents Index (SCI) of fish reared on different hatchery feeds over the course of wild caging trials: (a) overall and (b) over time (± SEM). Letters indicate significant differences between feed types (P< 0.05, 0.01, and 0.001 for brine shrimp-, pellet- and amphipod-reared versus wild fish, respectively). Numbers above error bars indicate the number of fish examined per time period. Duration post-release labels: 1d = up to 1 d, 1w = from 1 d to 1 wk, 2w = approximately 2 wk, and 1m = approximately 1 mo post-cage release. * and ** denote significant differences within feed types from values detected on Day 1 at $P < 0.05$ and 0.01, respectively.

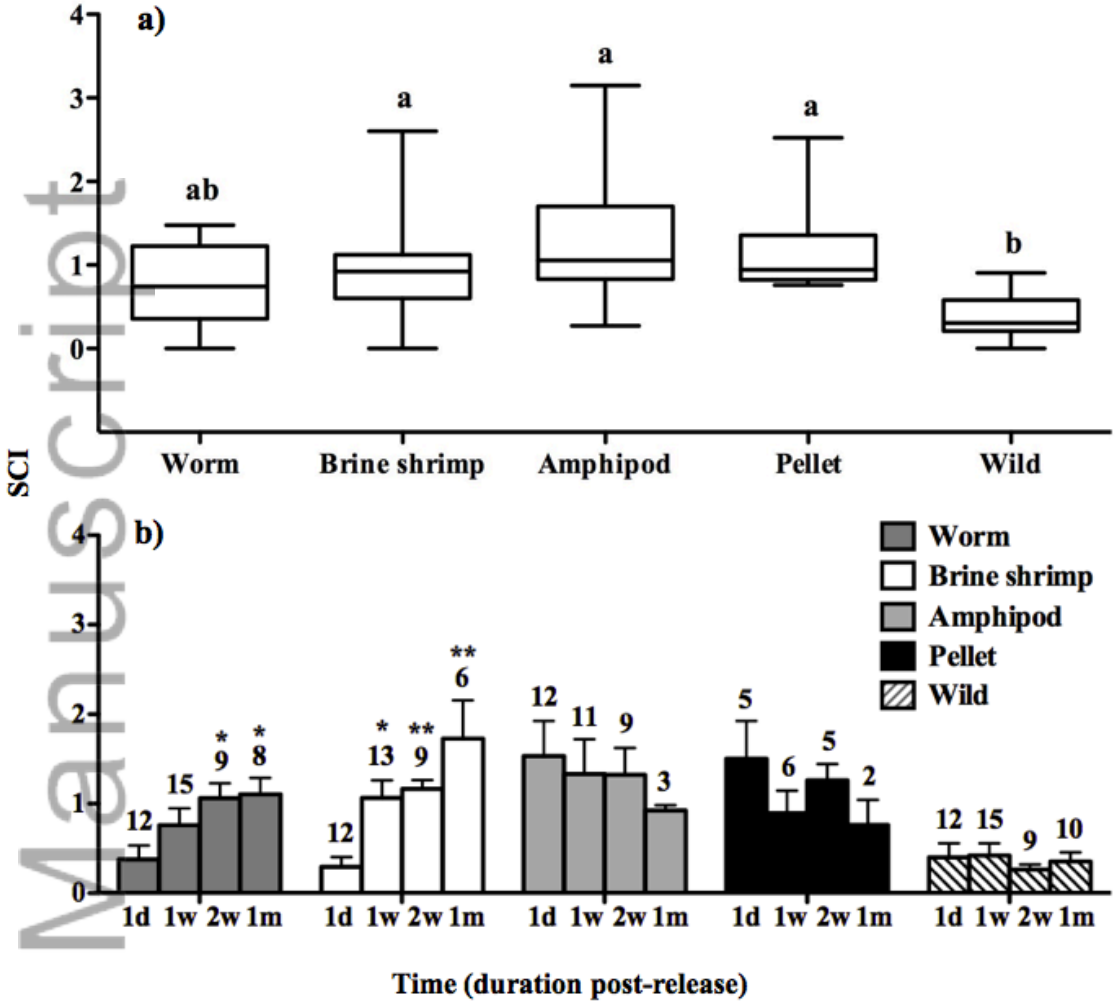

FIGURE 6. Dietary importance as indicated by Index of Relative Importance (IRI) for caged fish reared on different hatchery feeds overall and over time. Numbers above columns indicate the number of fish examined per time period. Duration post-release labels: 1d = up to 1 d, 1w = from 1 d to 1 wk, 2w = approximately 2 wk, and 1m = approximately 1 mo post-cage release.

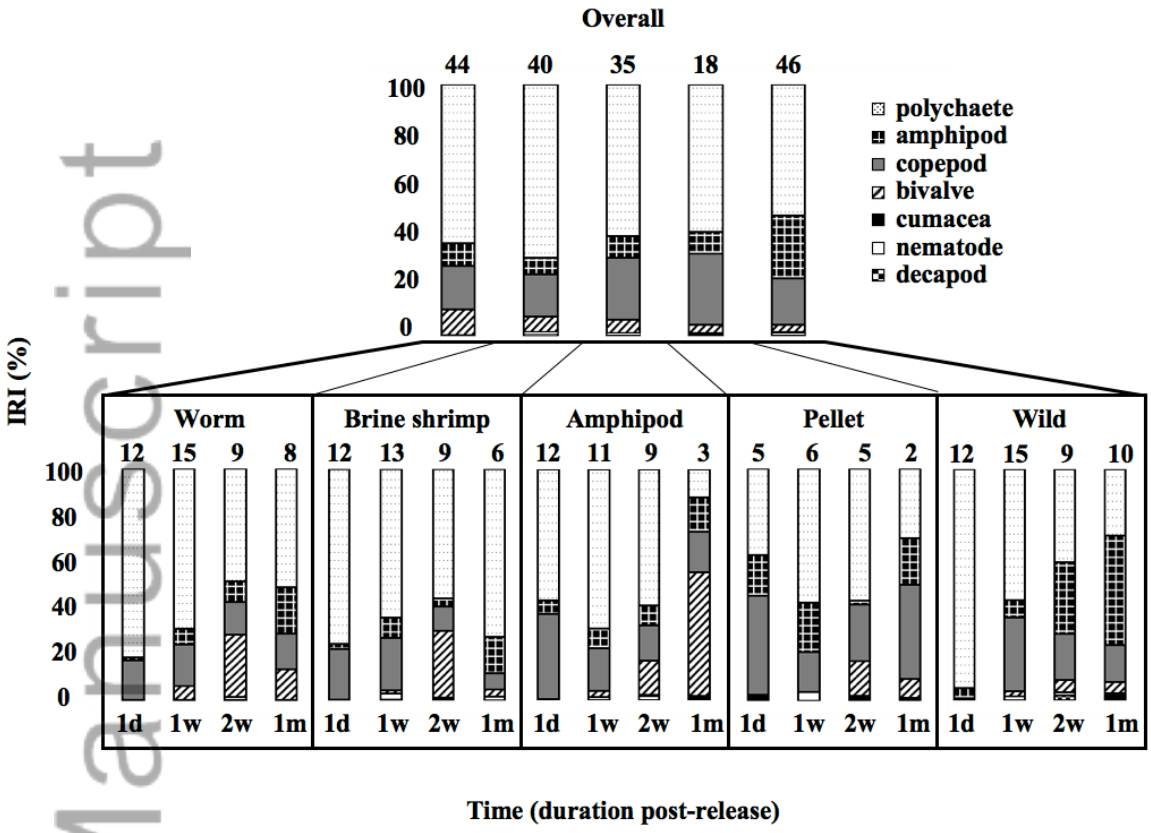

FIGURE 7. Nucleic acid measures for fish reared on different hatchery feeds, cage released, and retrieved > 3 d post release: over time (a, c, e) and overall (b, d, f). Each circular marker represents the mean of a feed type per day. Letters above error bars indicate significant differences between feed types ($P < 0.05$). Asterisk (*) indicates a slope significantly different from zero. Wt = weight.