

Measuring fish condition: an evaluation of new and old metrics for three species with contrasting life histories

Mark J. Wuenschel, W. David McElroy, Kenneth Oliveira, and Richard S. McBride

Abstract: Measuring fish condition should link ecosystem drivers with population dynamics, if the underlying physiological basis for variations in condition indices are understood. We evaluated traditional (K , K_n , hepatosomatic index, gonadosomatic index, energy density, and percent dry weight of muscle (%DWM) and liver (%DWL)) and newer (bioelectrical impedance analysis (BIA) and scaled mass index (SMI)) condition indices to track seasonal cycles in three flatfishes — winter flounder (*Pseudopleuronectes americanus*; three stocks), yellowtail flounder (*Limanda ferruginea*; three stocks), and summer flounder (*Paralichthys dentatus*; one stock) — with contrasting life histories in habitat, feeding, and reproduction. The %DWM and %DWL were good proxies for energy density ($r^2 > 0.96$) and more strongly related to K , K_n , and SMI than to BIA metrics. Principal component analysis indicated many metrics performed similarly across species; some were confounded by size, sex, and maturity along PC1, while others effectively characterized condition along PC2. Stock differences were along PC1 in winter flounder, reflecting different sizes across stocks, whereas in yellowtail flounder differences occurred along PC2 related to condition. These comparisons, within and across species, highlight the broad applicability of some metrics and limitations in others.

Résumé : La mesure de la condition des poissons devrait permettre de relier des facteurs écosystémiques à la dynamique des populations, si tant est que les fondements physiologiques des variations d'indices de condition sont bien compris. Nous avons évalué des indices de condition traditionnels (K , K_n , indice hépatosomatique, indice gonadosomatique, densité énergétique et pourcentages massiques à l'état sec des muscles (%MSM) et du foie (%MSF)) et plus récents (analyse de l'impédance bioélectrique (AIB) et indice de masse proportionnelle (SMI)) pour cerner les cycles saisonniers touchant à l'habitat, l'alimentation et la reproduction chez trois poissons plats, la plie rouge (*Pseudopleuronectes americanus*; trois stocks), la limande à queue jaune (*Limanda ferruginea*; trois stocks) et le cardeau d'été (*Paralichthys dentatus*; un stock), présentant des cycles biologiques différents. Le %MSM et le %MSF sont de bonnes variables substitutives de la densité énergétique ($r^2 > 0,96$) et sont plus fortement reliés aux K , K_n et SMI qu'aux paramètres de l'AIB. L'analyse en composantes principales indique une performance semblable de nombreux paramètres pour les trois espèces, l'effet de certains étant masqué par la taille, le sexe et la maturité le long de PC1, alors que d'autres caractérisent effectivement la condition le long de PC2. Les différences entre les stocks se situent le long de PC1 pour la plie rouge, reflétant une variation des tailles entre les stocks, alors que, pour la limande à queue jaune, les différences se manifestent le long de PC2, reliées à la condition. Ces comparaisons au sein d'espèces et entre elles font ressortir l'applicabilité large de certains paramètres et les limites d'autres paramètres. [Traduit par la Rédaction]

Introduction

The physiological health and energetic status of fishes is increasingly evaluated for a broad range of purposes: as a proxy for reproductive output (Marshall et al. 1999, 2003, 2006), to assess responses to biological interactions (Marshall et al. 2004; Cade et al. 2008), ecosystem change (Choi et al. 2004), as prey to higher trophic levels (Renkawitz et al. 2015), to categorize life history types within a population (Larsen et al. 2017), and to inform population status for management (Blackwell et al. 2000; Brosset et al. 2017; Morgan et al. 2018) and conservation (Stevenson and Woods 2006). Depending on the intended purpose (e.g., single species energy content and reproductive potential or multispecies ecosystem indicators), the desired characteristic of the metric chosen will vary. The concept of overall “health” of an organism is somewhat ambiguous and is considered to be an integration of many factors acting at the suborganism level that are more clearly defined and quantifiable (e.g., fat content, RNA–DNA, hemoglobin

concentration, etc.). The goal of defining the “best” states of condition in individuals and populations, and deviations from this optima, has been a pursuit of fisheries biologists for many years, and approaches can generally be classified as either morphophysiological (e.g., weight at a given length, hereinafter referred to as morphological) or physiological–biochemical (Shulman and Love 1999). To be useful, methods should (i) characterize functional features of organisms or populations, (ii) encompass the range of variability in the process examined, (iii) be representative of the population, and (iv) be easily measured under field conditions (Shulman and Love 1999). While physiological–biochemical methods have generally been more informative, they require more effort and are impractical for field sampling and large sample sizes. Numerous methods have been proposed to relate the physiological health (state of wellbeing, condition) of fishes based on physiological–morphological characteristics, with the premise that heavier or fatter is better (Le Cren 1951; Ricker 1975; Hayes

Received 26 February 2018. Accepted 27 July 2018.

M.J. Wuenschel and R.S. McBride. Northeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 166 Water Street, Woods Hole, MA 02543, USA.

W.D. McElroy. Integrated Statistics Inc., Woods Hole, MA 02543, USA. Under contract to: Northeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 166 Water Street, Woods Hole, MA 02543, USA.

K. Oliveira. Department of Biology, University of Massachusetts Dartmouth.

Corresponding author: Mark J. Wuenschel (email: Mark.Wuenschel@noaa.gov).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from [RightsLink](https://www.elsevier.com/locate/permissions).

and Shonkwiler 2001). The morphological approach offers ease of collection, but still needs to be validated to some functional feature (e.g., energy content) and often fails to accurately reflect nutritional condition of individual fishes.

The nondestructive nature of morphological indices is appealing, especially if they can be related to variables such as fat or energy content that are much more difficult to measure. Simple metrics such as Fulton's K relate the weight of an individual to that predicted from the cube of their length: $K = W/L^3$. Though Fulton's K has been in use for many years (Nash et al. 2006), problems related to the assumption of weight scaling as a cube of length have been demonstrated (Cone 1989). Since the exponent of the weight-length relation deviates from 3 in most species, the calculated Fulton's K is dependent on size, invalidating comparison of values from samples or individuals of different lengths. The relative condition factor (K_n) does not assume a length exponent, but fits a length-weight relationship to the available data (sample or population). Individual K_n is then calculated as the observed weight/predicted weight based on length (Le Cren 1951); thus, a value of 1 indicates "average" condition. One disadvantage of K_n is that given its "relative" nature, when additional data are added a new predictive regression is required, which can change prior individual K_n values (e.g., with additional sampling or data, what was considered "average" condition may now be shown to be above or below average). This "internal" (study-specific) aspect of calculating K_n also makes it difficult to compare values across studies. The relative weight index (W_r ; Blackwell et al. 2000) addresses this reliance on an internally estimated length-weight relation by using a standard equation for each species (i.e., a "global mean") and allows cross-study comparison. W_r has been applied more commonly for freshwater species, where it may be impractical to develop length-weight relations for every pond or lake. More recently, Peig and Green (2009) proposed use of a scaled mass index (SMI), which is based on the central principle of scaling. Compared with other (more traditional) morphometric condition indices, the SMI was shown to be a better predictor of fat and energy reserves in a variety of organisms (small mammals, birds, and snakes) and has since been applied to a broader range of animals, including amphibians (MacCracken and Stebbings 2012) and fishes (Camizuli et al. 2014; Maceda-Veiga et al. 2014; Morita et al. 2015; Masse et al. 2016).

In addition to advances in analytic methods for morphological condition indices, new technologies have emerged and have been applied to quantify physiological condition, including total body electrical conductivity (TOBEC; Scott et al. 2001), bioelectrical impedance analysis (BIA; Cox and Hartman 2005), and the fatmeter (Crossin and Hinch 2005; Davidson and Marshall 2010; Schloesser and Fabrizio 2017). Both TOBEC and BIA operate on the principle that the conductivity of an organism is determined mainly by its lean tissues (Scott et al. 2001). BIA has been applied successfully to estimate whole body fat and energy in fish in several studies (Cox and Hartman 2005; Cox and Heintz 2009; Hafs and Hartman 2011), but less successfully in other studies (Pothoven et al. 2008; Garner et al. 2012; Klefoth et al. 2013; Dibble et al. 2017).

Regardless of condition index chosen, there is a need to relate any index at different stages in a fish's life to standard physiological variables (e.g., fat or energy density; Davidson and Marshall 2010; Schloesser and Fabrizio 2017). For example, condition indices, whether morphometric or derived from physical (bioelectrical) properties, may reflect different aspects of an individual's physiology and may be affected differently by both the reproductive state (on an annual cycle; Robards et al. 1999) and reproductive mode of a given species. This study focuses on three species with different life histories, particularly in terms of reproduction, which is energetically demanding and likely to affect fish condition.

Teleosts display a wide diversity in reproductive modes (Smith and Wootton 2016) and energy allocation strategies for reproduc-

tion that can be generalized along a continuum from capital to income breeding types that may be a response to seasonal cycles of food availability (McBride et al. 2015). The high fecundity of many fishes (as compared with other vertebrates) requires that fishes devote a large percentage of surplus energy to reproduction. Even within species that spawn multiple seasons in their lifetime (i.e., iteroparous), ovary mass may exceed 40% of total body mass in the period leading up to spawning. Such substantial buildup and release of gametes is energetically demanding and often requires mobilization (depletion) of stored energy from other parts of the body (e.g., soma, liver). Therefore, in addition to the gonad, the soma, liver, and viscera or mesentery may also fluctuate in response to reproductive condition. Since some of these body components function as energy storage, the seasonal cycles of buildup and depletion is often temporally offset from that of reproductive output. These internal dynamics within individual fish can confound efforts to assess condition from measures of whole body weight. Therefore, even if the weight of the gonad is accounted for in measures of condition based on weight, the seasonal energetic cycle related to spawning (and (or) feeding) may not be fully accounted for in analyses of condition from length-weight data.

Here, we measured biochemical measures of condition, as well as morphological and electrical indices, for three species. The three species — winter flounder (*Pseudopleuronectes americanus*), yellowtail flounder (*Limanda ferruginea*), and summer flounder (*Paralichthys dentatus*) — were chosen because of their contrasting habitat use, feeding modes, and reproductive patterns (Table 1). In terms of habitats, winter flounder is structured as three populations that reside in estuaries, shelf areas, and offshore banks from New Jersey northward; yellowtail flounder is structured in three populations that reside in the New England offshore shelf areas and offshore banks; and summer flounder is a single population distributed in coastal waters and along the shelf in the warm temperate zone, from the Carolinas to Georges Bank. Stock-specific growth, maturity, and fecundity have been reported in both winter flounder (McBride et al. 2013; McElroy et al. 2013) and yellowtail flounder (Klein-MacPhee 2002a; McElroy et al. 2016). Winter flounder is an example of an extreme capital breeder that ovulates an entire annual fecundity at once (i.e., total spawner; Press et al. 2014; McBride et al. 2015; McElroy et al. 2013). Winter flounder spawn benthic eggs and are also unusual in that they spawn in estuaries, but also in nearshore waters (DeCelles and Cadrin 2011; Fairchild et al. 2013) and on Georges Bank (Klein-MacPhee 2002a). Yellowtail flounder is another boreal-temperate species, similar to winter flounder. Although yellowtail flounder fecundity is determinate and oocyte development is group-synchronous, they ovulate and release pelagic eggs in batches and remain in offshore shelf waters. Both winter and yellowtail flounder are benthivores (Link et al. 2002; Smith and Link 2010), feeding primarily on invertebrates (polychaetes, gammarids, and anthozoans). Summer flounder also inhabits coastal-shelf habitats, but has a more southerly distribution (temperate-subtropical) and with extensive seasonal movements from coastal waters to the outer continental shelf (Klein-MacPhee 2002b). In contrast with winter and yellowtail flounder, summer flounder have asynchronous oocyte development, continuing to recruit and produce batches of eggs throughout a protracted spawning season; therefore, annual fecundity is indeterminate (Merson et al. 2000). This reproductive pattern is characteristic of income breeders (McBride et al. 2015). Summer flounder attain larger sizes, exhibit more pronounced sex-dimorphic growth, and feed on higher trophic-level prey, including fish and squid (Link et al. 2002; Smith and Link 2010; Wuenschel et al. 2013a).

Consequently, winter and summer flounder are at opposite ends of the reproductive spectrum in regard to energy allocation (capital, total spawner versus income, batch spawner), with yellowtail flounder intermediate between these two ends of this

Table 1. Summary of life history characteristics for the three flatfishes studied.

	Winter flounder	Yellowtail flounder	Summer flounder
Spawning pattern	Total spawner	Batch spawner	Batch spawner
Spawning location	Estuaries and ocean	Ocean	Ocean
Oocyte development	Group-synchronous	Group-synchronous	Asynchronous
Egg type	Benthic	Pelagic	Pelagic
Fecundity type	Determinate	Determinate	Indeterminate
Feeding	Benthivore	Benthivore	Piscivore
Energy allocation for reproduction ^a	Capital	Capital	Income–capital
Feeding versus reproduction ^b	Rester	Rester	Indeterminate
Median size (L_{50} ; cm) at maturity ^c	SNE: M = 29; F = 30 GOM: M = 28; F = 26 GB: M = 26; F = 34	SNE: M = 20; F = 26 GOM: M = 27; F = 27 GB: M = 21; F = 26	M 25; F 28
Median age (A_{50} ; years) at maturity ^c	SNE: M = 3.3; F = 2.6 GOM: M = 3.3; F = 3.9 GB: M = 1.9; F = 3.1	SNE: M = 1.8; F = 1.6 GOM: M = 2.6; F = 2.6 GB: M = 1.3; F = 1.8	M = 2; F = 2.5
Maximum size ^d and age ^e	64 cm; 3.6 kg; 15 years	64 cm; 1.5 kg; 17 years	94 cm; 12 kg; 12 years
Seasonal movements ^e	Limited (10s of km)	Limited (10s of km)	Extensive (100s of km)

Note: Stock regions (GB, Georges Bank; GOM, Gulf of Maine; SNE, southern New England) and sex (F, female; M, male) are shown.

^aSensu McBride et al. 2015.

^bSensu Link and Burnett 2001.

^cWinton et al. 2014; O'Brien et al. 1993.

^dRobins and Ray 1986.

^eKlein-MacPhee 2002a, 2002b.

spectrum. Therefore, variation across seasons and among species provides contrast in multiple measures of physiological condition. The objectives of this study were to measure a suite of morphological and physiological–biochemical properties of these three species with different energy allocation strategies to determine inter-relations between the various measures of condition and therefore the suitability of easily measured “proxies” to accurately estimate physiological condition. The diversity of life history traits exhibited by these three species encompasses that for a wide range teleosts, so the conclusions drawn from them should be broadly applicable.

Methods

Sample sources

The collections used here are part of ongoing efforts to investigate and monitor the reproductive biology of three flatfish species — winter flounder, yellowtail flounder, and summer flounder — and some aspects of this research have been reported previously (McElroy et al. 2013, 2016; Press et al. 2014). The present analysis includes samples collected using bottom trawls from several sources: the Northeast Fishery Science Center (NEFSC) Northeast Cooperative Research Branch's (CRB) Study Fleet and other CRB field research, the Massachusetts Department of Fish and Game Division of Marine Fisheries (MADMF) bottom trawl survey, the NEFSC Ecosystems Surveys Branch bottom trawl survey, and the University of Rhode Island Graduate School of Oceanography (URI) bottom trawl survey (summarized in Table 2). These sources allowed coverage of portions of the Gulf of Maine, Georges Bank, and southern New England stocks of winter and yellowtail flounder and the single stock of summer flounder. Immediately after capture, fish were iced down and transported to the laboratory for processing. Intensive monthly sampling of all three species (from seven fishery stocks) was conducted from December 2009 until spring 2011, after which collections were focused on the reproductive season of each stock to conserve resources. Summer flounder collections ceased after winter 2013, but sampling for the other species continued (2014–2016), targeted toward the period just prior and through peak spawning for each stock.

Sample processing

First, BIA was performed on fish in the lab using the Quantum X Body Composition analyzer (RJL Systems Inc.). Electrical resistance and reactance between pairs of electrodes placed in the dorsal

musculature of the eyed side were measured while the fish was on a nonconductive measuring board with moveable electrode holders (Fitzhugh et al. 2010). Each electrode pair included a signal-emitting and a signal-detecting electrode spaced 10 mm apart. Leads from the device were attached directly to steel dissecting pins set to penetrate a depth of 5 mm. At the start of each sampling session, the resistance and reactance of a standard resistor (500 ohms) connecting directly to the electrodes were measured to ensure all electrical connections were correct and readings were within tolerance (resistance, 500.0 ± 5.0 ohms; reactance 0.0 ± 3.0 ohms). The following anatomical landmarks were used for electrode placement: anterior electrode placed dorsal to the pectoral fin, midway between lateral line and dorsal margin; posterior electrode set aligned with dorsal fin insertion at the caudal peduncle, midway between the lateral line and dorsal margin. From these measured electrical properties, additional bioelectrical properties were calculated (Table 3) following Cox and Hartman (2005), Hafs and Hartman (2011), and Stolarski et al. (2014).

The following measures were obtained: total length (± 1 mm), total weight (± 0.1 g), sex, maturity, liver weight (± 0.001 g), and gonad weight (± 0.001 g). Maturity was assessed macroscopically following the criteria outlined in Burnett et al. (1989), O'Brien et al. (1993), and McBride et al. (2013) and initially assigned to six classes: immature, developing, ripe, running ripe, spent, and resting. For the present analysis, ripe and running ripe were combined as ripe. Gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated as $100 \times ([\text{gonad or liver}] \text{ weight} / (\text{body weight} - [\text{gonad or liver}] \text{ weight}))$, respectively (Wootton 1990). For a subset of individuals of each species, additional samples of liver (mean = 7.194, standard deviation (SD) = 3.741 g wet weight) and epaxial muscle (mean = 7.617, SD = 3.865 g wet weight) tissue were removed to determine the water content (and percent dry weight).

Liver and epaxial muscle tissue samples were dried at 60 °C to a constant weight, and the percent dry weight (%DW; unitless) of muscle and liver tissue was calculated as $\%DW = 100 \times (\text{dry weight} / \text{wet weight})$. A subset of these samples ($n = 266$) across a range of seasons, sex, maturity classes, and percentages of dry weight for each tissue type and species was selected for proximate composition analysis. Proximate composition of subsamples of dried tissue were analyzed gravimetrically (following Morley et al. 2012), using Soxhlet extraction apparatus with petroleum ether as the solvent to determine lipid content, and then re-dried to a constant

Table 2. Summary of numbers and sizes of fish examined for the present analysis.

Year	Source	Winter flounder			Yellowtail flounder			Summer flounder		
		N	Mean TL (min.–max.)	Months sampled	N	Mean TL (min.–max.)	Months sampled	N	Mean TL (min.–max.)	Months sampled
2009	CP	63	324.5 (226–485)	12	20	299.2 (237–348)	12	32	424.8 (232–679)	12
	SF	13	347.8 (312–401)	12	44	376.5 (332–432)	12	29	439.3 (384–591)	12
		76	328.5 (226–485)	12	64	352.3 (237–432)	12	61	431.7 (232–679)	12
2010	BG	10	336.1 (215–426)	10	31	379.1 (277–432)	10	2	457.0 (454–460)	10
	CP	148	351.1 (155–548)	3, 4, 10, 11	157	327.1 (202–443)	3, 4, 10, 11	23	426.0 (256–668)	4
	MA	75	210.1 (90–398)	5, 9	31	309.5 (210–383)	5, 9	15	317.5 (216–353)	9
	RI	43	243.3 (90–372)	1–4				7	314.3 (290–337)	8
	SF	674	335.6 (155–525)	1–3, 5–12	693	366.8 (232–487)	1–3, 5–12	376	491.1 (242–736)	1–3, 5–12
		950	324.0 (90–548)	1–12	912	358.5 (202–487)	1–12	423	478.3 (216–736)	1–12
2011	MA	101	256.1 (76–441)	5	86	299.4 (118–426)	5	3	271.0 (257–283)	5
	SF	348	361.6 (220–523)	1–5	431	366.3 (240–475)	1–6	513	469.5 (295–730)	1–5, 9–11
		449	337.9 (76–523)	1–5	517	355.1 (118–475)	1–6	516	468.3 (257–730)	1–5, 9–11
2012	MA	125	297.0 (137–452)	5	7	350.0 (314–433)	5			
	SF	268	374.9 (281–490)	1–5	270	368.7 (317–464)	3–5	399	496.6 (361–723)	3–4, 9–11
		393	350.1 (137–490)	1–5	277	368.2 (314–464)	3–5	399	496.6 (361–723)	3–4, 9–11
2013	MA	37	207.2 (101–349)	5	19	317.6 (219–427)	5			
	SF	260	375.9 (291–491)	1–5	379	368.6 (319–448)	3–5	87	516.3 (374–718)	3–4
		297	354.9 (101–491)	1–5	398	366.1 (219–448)	3–5	87	516.3 (374–718)	3–4
2014	SF	267	369.5 (295–493)	1–4	289	373.5 (304–457)	3–6			
		267	369.5 (295–493)	1–4	289	373.5 (304–457)	3–6			
2015	SF	179	363.4 (281–461)	1–4	389	377.4 (307–462)	2–5			
		179	363.4 (281–461)	1–4	389	377.4 (307–462)	2–5			
2016	SF	239	366.9 (296–469)	1–4	306	365.6 (299–502)	2–5			
		239	366.9 (296–469)	1–4	306	365.6 (299–502)	2–5			
All		2850	343.4^a (76–548)		3152	364.0^a (118–502)		1486	480.0^a (216–736)	

Note: CP, Northeast Fisheries Science Center (NEFSC) Cooperative Research Branch (CRB) gear study; SF, CRB Study Fleet; BG, NEFSC Ecosystems Surveys Branch bottom trawl survey; MA, Massachusetts Department of Fish and Game Division of Marine Fisheries bottom trawl survey; RI, University of Rhode Island Graduate School of Oceanography bottom trawl survey. Totals (within each year and across all years) are indicated in bold.

^aMean values used as L_0 in calculation of scaled mass index.

weight to determine lipid-free weight. The lean (lipid-free) sample was then combusted in a muffle furnace at 500 °C for 5 h, cooled to ambient temperature, and re-dried to constant weight to determine ash content. Preliminary analysis indicated 5 h combustion time was sufficient for the size and composition of these samples (K. Oliveira, unpublished data). The lean tissue weight was assumed to be protein; phospholipids and carbohydrates are minor fractions in fish tissue (Love 1980). Liver energy density (LED; $\text{kJ}\cdot\text{g}^{-1}$) and muscle energy density (MED) were calculated per gram wet weight using the energy equivalent values for lipid ($39.54 \text{ kJ}\cdot\text{g}^{-1}$) and protein ($23.64 \text{ kJ}\cdot\text{g}^{-1}$) (Henken et al. 1986; Morley et al. 2012). Total liver energy (TLE) was then calculated by multiplying the energy density (measured directly or estimated from ED versus %DW relationships) by the total liver weight.

Morphometric condition indices

Fulton’s K of individual fish was calculated as $K = W_i/L_i^3$, where W_i and L_i are the observed individual fish weight and length, respectively. Relative condition (K_n) was calculated as $K_n = \text{observed weight}/\text{predicted weight}$ (i.e., W_i/aL_i^b), where W_i and L_i are as above, and a and b are species-specific regression constants obtained from the overall length–weight relationships using pooled data (Table 4). Because sample coverage varied over the course of the study (with later years focused only on the few months leading up to spawning), we developed length–weight equations from the first ~2 years of collections when (near) monthly samples were obtained. This approach captured the seasonal cycle in weight at length (albeit for only one to two spawning seasons) while reducing potential bias due to the predominance of fish in prespawning condition in the more recent years.

We calculated the SMI following Peig and Green (2009), who applied the Thorpe–Leonart model of scaling (Leonart et al. 2000) to length–mass:

$$\text{SMI} = M_i \left(\frac{L_0}{L_i} \right)^{b_{\text{SMA}}}$$

where M_i is body mass; L_i is length of individual i ; b_{SMA} is the scaling exponent obtained from the species-specific standardized major axis regression of $\ln M$ versus $\ln L$; L_0 is an arbitrary reference length chosen for standardization; and SMI is the predicted body mass of individual i standardized to L_0 (Table 4). For the reasoning above for K_n , we used data from the first ~2 years of sampling to estimate length–weight parameters. As in other studies, we set L_0 to the arithmetic mean length sampled for each species.

Data analysis

The significance of seasonal variation in physiological measures and morphological condition indices was assessed using the non-parametric Kruskal–Wallis test, since the monthly data had unequal variances (Levene’s test, $P < 0.05$) and (or) non-normal distributions (Shapiro–Wilk, $P < 0.05$) in all cases. Pearson correlation coefficients between the various morphological, physiological, and biochemical measures (summarized in Table 3) were calculated and visualized for each species using the corrplot package in R (Wei and Simko 2016). Linear regressions of the relationships between tissue (muscle and liver) energy density and tissue percent dry weight were developed for each species.

Multiple morphometric and physiological measures were collected on each specimen, increasing the likelihood of (i) redundant (covarying, correlated) variables and (ii) high type I error if all combinations were analyzed. Therefore, to evaluate relationships between the multiple morphological and physiological measures recorded for each specimen (i.e., multivariate data), we applied ordination methods to reduce the numbers of axes to capture the

Table 3. Summary of biological variables, morphometric indices, and bioelectrical and biochemical variables analyzed.

Measure	Abbreviation	Equation
Biological variable		
Total length (mm)	TLENGTH	—
Total weight (g)	TWEIGHT	—
Gonad-free fish weight (g)	GFWEIGHT	TWEIGHT - GWEIGHT
Gonad weight (g)	GWEIGHT	—
Liver weight (g)	LWEIGHT	—
Gonadosomatic index	GSI	$100 \cdot [GWEIGHT / (TWEIGHT - GWEIGHT)]$
Hepatosomatic index	HSI	$100 \cdot [LWEIGHT / (TWEIGHT - LWEIGHT)]$
Morphometric condition index		
Fulton's K	K	$10^5 \cdot TWEIGHT / TLENGTH^3$
Relative condition	K_n	$TWEIGHT / \text{predicted mass}$
Scaled mass index	SMI	$M_i \left(\frac{L_0}{L_i} \right)^{b_{SMA}}$
Bioelectrical impedance analysis		
Resistance (ohms) ^a	RESIS	—
Reactance (ohms) ^a	REACT	—
Resistance in series (ohms)	RS	$DL^2 / RESIS$
Reactance in series (ohms)	XC	$DL^2 / REACT$
Resistance in parallel (ohms)	RP	$DL^2 / [RESIS + (REACT^2 / RESIS)]$
Reactance in parallel (ohms)	XCP	$DL^2 / [REACT + (RESIS^2 / REACT)]$
Capacitance (picofarads)	CPF	$DL^2 / \{ [1 / (2 \cdot \pi \cdot 50000 \cdot RESIS)] (1 \cdot 10^{12}) \}$
Impedance in series (ohms)	ZS	$DL^2 / (RESIS^2 + REACT^2)^{0.5}$
Impedance in parallel (ohms)	ZP	$DL^2 / [RESIS \cdot REACT / (RESIS^2 + REACT^2)^{0.5}]$
Phase angle (degrees)	PA	$\text{Arctan}(REACT / RESIS) \cdot 180 / \pi$
Standardized phase angle (degrees)	PADL	$DL \cdot [\text{Arctan}(REACT / RESIS) \cdot 180 / \pi]$
Body mass index (ohms)	BMI	$\{ [(RESIS^2 + REACT^2)^{0.5}] \cdot TWEIGHT \} / DL^2$
Biochemical		
Percent dry weight of muscle	%DWM	$100 \cdot (\text{dry weight} / \text{wet weight})$
Percent dry weight of liver	%DWL	$100 \cdot (\text{dry weight} / \text{wet weight})$
Total liver energy (kJ)	TLE	$LWEIGHT \cdot \text{liver energy density}$

Note: See text for explanation of predicted mass used to calculate K_n ; DL is detector length (distance between electrode pairs). Units for each measure are indicated in parentheses if not unitless.

^aMeasured by Quantum X.

Table 4. Summary of nonlinear regression models to determine parameters for calculation of K_n and standardized major axis regression model for parametrization of scaled mass index (SMI).

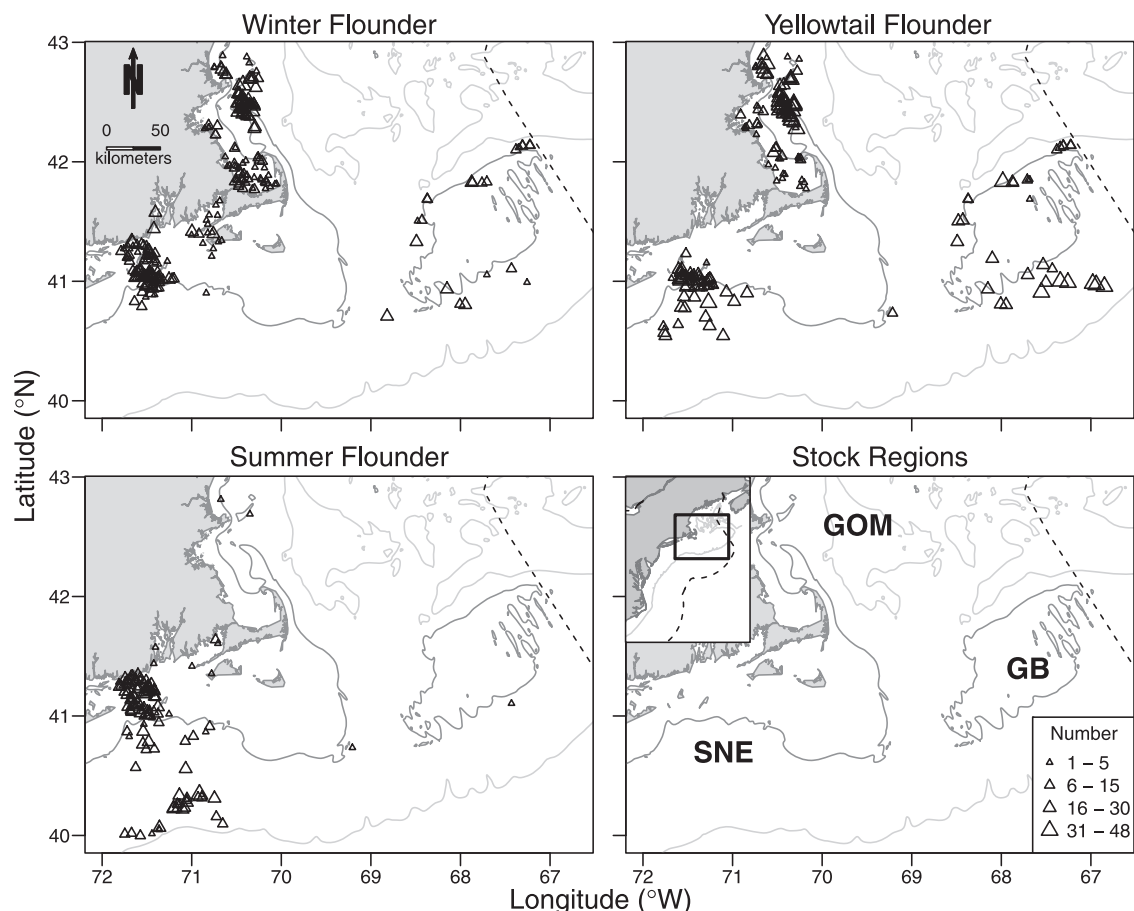
(a) K_n					
		Intercept	Slope		
	n	a	b		
Winter flounder	1732	1.340×10^{-5} (1.393×10^{-6})	2.989 (1.726×10^{-2})		
Yellowtail flounder	1522	2.749×10^{-6} (4.746×10^{-7})	3.192 (2.895×10^{-2})		
Summer flounder	1079	1.776×10^{-6} (1.758×10^{-7})	3.286 (1.544×10^{-2})		
(b) SMI					
		Intercept	b_{SMA}	L_0	r^2
Winter flounder	1732	-11.738 (-11.846, -11.630)	3.0765 (3.0578, 3.0952)	343.4	0.983
Yellowtail flounder	1522	-12.928 (-13.155, -12.704)	3.2127 (3.1745, 3.2514)	364.0	0.943
Summer flounder	1079	-12.955 (-13.082, -12.829)	3.2402 (3.2197, 3.2609)	480.0	0.989

Note: In parentheses are standard error for K_n parameter estimates and 95% CI for SMI parameter estimates.

main trends in the data. We used principal component analysis (PCA) to explore patterns between morphological, biological, and physiological variables measured for each species. This method is descriptive in nature and is considered an appropriate method to explore structure in the observed variation in the data and allowed us to determine what aspects of "condition" were captured by each of the indices, thus serving as a validation tool without any a priori assumptions regarding response versus explanatory

variables. The PCA was run using the correlation matrix of the scaled data (unit variance) for all of the continuous variables, and sex, reproductive phase, and stock were included as supplemental (categorical) variables. Individuals missing data for any of the variables were removed prior to analysis. For each species, PCA was performed using the FactoMineR package (Le et al. 2008) in R statistical software, with results visualized using Factoextra (Kassambara and Mundt 2016). The categorical variables are not

Fig. 1. Distributions of sample locations for winter, yellowtail, and summer flounder on the Northeast US continental shelf. Symbols are scaled to sample sizes from each location, and stock regions are indicated. The dark and light gray lines indicate the 50 and 200 m isobaths, respectively, and the dashed line indicates the Exclusive Economic Zone (EEZ) boundary. Stock regions (Georges Bank, GB; Gulf of Maine, GOM; southern New England, SNE) apply to winter and yellowtail flounder.



used in forming the principal components (PCs); therefore, the unbalanced nature of our sampling with respect to sex, reproductive phase, or stock did not introduce bias. The categorical variables were “mapped” onto the PCA space, and categories that were characteristic of each PC were identified (dimdesc function in FactoMineR). The number of PCs retained was based on visual evaluation of the scree plots of variation explained and interpretability of the loadings.

Results

Fish were analyzed from a broad geographic region, encompassing portions of three fishery stock areas for winter flounder and yellowtail flounder; however, sample sizes were lower from Georges Bank for both (Fig. 1). Summer flounder samples were almost entirely from the southern New England portion of the stock, but were from both inshore in summer and deeper offshore areas during the colder months.

Condition metrics

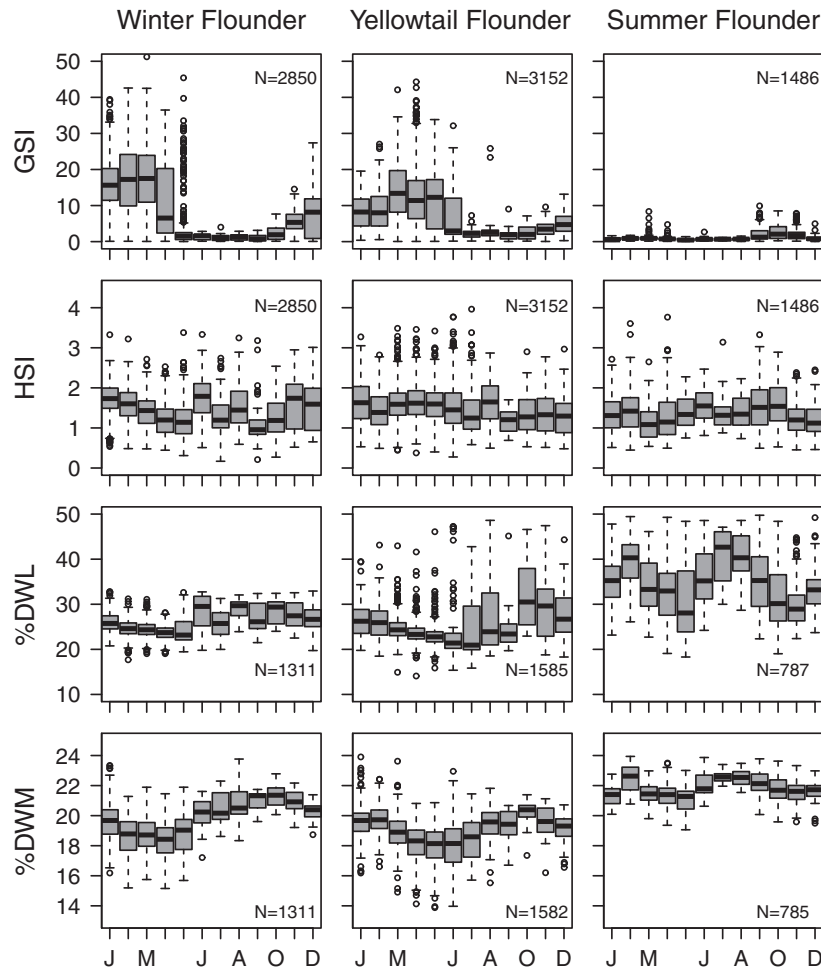
The physiological measures varied across species and seasons, reflecting differential reproductive modes and energy allocation strategies (Fig. 2). In all cases, observed variation in physiological measures across months was significant (Kruskal–Wallis, $P < 0.001$; see online supplemental data, Table S1¹). Winter flounder showed extreme variation in GSI through the year, approaching 40% just

prior to and during spawning in winter. Yellowtail flounder, a spring spawner, showed a notably large seasonal variation in GSI, but to a lesser degree than winter flounder. Summer flounder, primarily a fall spawner in this region, demonstrated the least magnitude variation in GSI, with values ranging from <2% in the nonspawning period to a maximum of <10% during spawning. The relative size (median 1%–2% of fish mass) and seasonal variation in size of the liver (HSI) was similar across species; however, the composition of the liver (%DWL) followed distinct seasonal patterns in all species, and summer flounder had 5% to 10% higher peak %DWL. Winter flounder exhibited less variation in %DWL than either other species with a range of only 10%–12%. Seasonal cycles in muscle composition (%DWM) were also evident for all three species, with winter and yellowtail flounder having stronger seasonal patterns, while summer flounder had higher %DWM and lower individual variation. Peaks in %DWM and %DWL occurred in the nonspawning period for winter and yellowtail flounder. Summer flounder exhibited two peaks for %DWM and particularly %DWL, one occurring in winter and one in summer.

The three morphometric condition indices generally captured seasonal changes in fish condition related to spawning seasonality, showing lowest values immediately following spawning and recovering thereafter (Fig. 3). As for physiological measures, in all cases the observed variation in morphometric condition indices across months, although subtle as compared with individual vari-

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2018-0076>.

Fig. 2. Monthly gonadosomatic index (GSI), hepatosomatic index (HSI), percent dry weight of liver (%DWL) and muscle (%DWM) of winter, yellowtail, and summer flounder. Box plots indicate median (line within each box), 25th and 75 percentiles (box limits), extremes of the data determined as $1.5 \times$ interquartile range (whiskers), and any outliers (circles). See Table 3 for variable descriptions.



ation, was significant (Kruskal–Wallis, $P < 0.001$; Table S1¹). The seasonal signal was strongest in winter flounder and weakest in summer flounder. For each species, the patterns observed for K and K_n were similar, while values of SMI indicated more extreme low values for winter and yellowtail flounder. During the spawning seasons for both winter and yellowtail flounder, there were more individuals identified as outliers with low SMI. The patterns in phase angle (PA) were more ambiguous and did not closely follow patterns in K , K_n , and SMI for winter and yellowtail flounder except for the low values in July for yellowtail flounder. For summer flounder, the PA followed the patterns in morphometric indices.

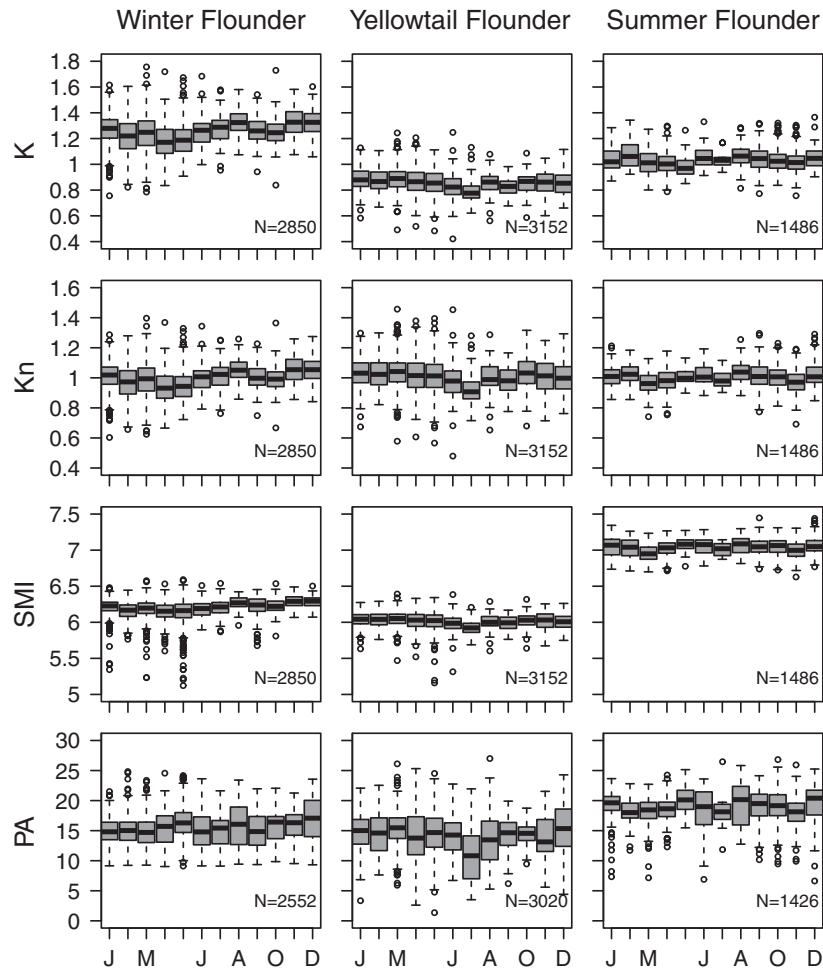
Overall, the observed seasonal patterns in both physiological and morphometric condition indices for winter and yellowtail flounder generally showed a single peak and valley, while in summer flounder there was indication of two peaks occurring through the year for many measures. Winter flounder exhibited larger variation than the other species in measures influenced by muscle mass, whereas yellowtail and summer flounder demonstrated larger variation in liver metrics than winter flounder. Within monthly summary box plots, variation in both physiological and condition indices was large, reflecting the diversity of sexes and reproductive phases sampled. This variation was greater in winter and yellowtail flounder, where the monthly aggregates include fish from multiple stocks with differing peak spawning periods (see Fig. S1¹).

Correlations

Correlations between physiological and morphometric condition indices within each species revealed several commonalities (Figs. 4, 5, 6). The three morphometric condition indices (K , K_n , SMI) had strong positive correlations with each other, except for SMI of summer flounder. In all species, both K and K_n had weak positive correlations with physiological measures, including %DWL, %DWM, TLE, GWEIGHT, LWEIGHT, GSI, and HSI (refer to Table 3 for terms). For SMI, correlations with these same physiological measures ranged from weakly positive in yellowtail, weakly positive and weakly negative in winter flounder, to no relation or strongly negative in summer flounder. The electrical properties calculated from BIA measures were strongly correlated to each other, but also were strongly correlated to measures of size (TLENGTH, TWEIGHT, GFWEIGHT) and properties that covaried with size (GWEIGHT, LWEIGHT). The %DWL and %DWM were generally correlated with each other, but none of the morphometric, biological, or BIA measures were strong predictors of %DW in these tissues. The TLE was strongly correlated to %DWL, HSI, and LWEIGHT.

For each species, strong relations were observed between the percent dry weight of liver and muscle and the energy content of those tissues (Fig. 7; Table 5). Analysis of variance (ANOVA) indicated a significant main effect of species on the relationships between energy density and %DWL ($P < 0.001$), but the interaction between species and %DWL was not significant ($P = 0.083$). Simi-

Fig. 3. Monthly morphometric indices (K , K_n , SMI) and phase angle (PA) of winter, yellowtail, and summer flounder. Box plots indicate median (line within each box), 25th and 75 percentiles (box limits), extremes of the data determined as $1.5 \times$ interquartile range (whiskers), and any outliers (circles). See Table 3 for variable descriptions. SMI for each species is calculated using $L_0 =$ mean size collected for each species.



larly, there was a significant species effect (ANOVA, $P < 0.001$) on the relationship between energy density of muscle and %DWM, but the interaction term was not significant ($P = 0.465$). Species-specific regressions are presented in Table 5. Despite differences in regression intercepts, the data largely overlapped across species; however, the range of observed values in %DWM differed; summer flounder %DWM was never observed below 18.8%.

PCA summaries

The PCA analyses indicated similar influence (i.e., redundancy) of several variables across species (Table 6). For each species the first PC was strongly correlated to direct measures of fish size (e.g., TLENGTH, TWEIGHT, GFWEIGHT, LWEIGHT, GWEIGHT) as well as size-dependent (i.e., not standardized to detector length) BIA measures (e.g., RS, CPF, ZS, RP, ZP, XC, and XCP; Figs. 8, 9, and 10; refer to Table 3 for terms). Across species, the second PC was generally related to various measures of condition (K , K_n , SMI, BMI, PA). The third and fourth PCs were significantly correlated with several variables; however, they generally explained low amounts of variation (5.7%–10.8%).

For winter flounder, the first PC axis explained 48.7% of the observed variance and was highly correlated to several measures associated with size (Table 6). The second PC explained an additional 17.6% and was influenced by the morphometric condition indices (K , K_n , SMI). The third PC explained 10.8% of the variation and was most related to three BIA measures (PADL, REACT, and PA). Finally, the fourth PC explained 7.2% of the variance and was

related to %DWM, %DWL, and GSI. Visualizations of data grouped by qualitative variables (reproductive phase, sex, stock) revealed the following: immature individuals were grouped on the left of PC1 (associated with smaller size), developing individuals were generally higher along PC2 (higher condition), while spent fish were lower on this axis (i.e., lower condition; Table 6; Fig. 8). Sex did not show as strong effects, but did show some influence related to fish size (PC1; i.e., higher values associated with the larger size reached by females). The stock effect was largely along PC1, driven by the larger size of the individuals in the GB stock (Fig. 11).

For yellowtail flounder, the first PC explained 42.1% of the overall variation and was also highly correlated to measures associated with size (Table 6). The second PC explained an additional 23.8% of the variation and was influenced primarily by morphometric and BIA condition metrics (K , K_n , SMI, BMI, PA, and PADL). The third PC explained an additional 10.8% of the variation, but except for PADL, the correlations with individual variables were weaker. The fourth PC explained 7.9% of the variation and was influenced by %DWL and GSI. Visualizations of the data by qualitative grouping variables revealed the following: immatures and males grouped to the left on PC1 (smaller sized) and spent fish were generally lower on PC2 (i.e., lower condition; Table 6; Fig. 9). Stock differences in yellowtail flounder were stronger along PC2 (i.e., condition) than PC1; the GB stock had more variable and lower condition, particularly for larger fish (higher PC1), and more SNE fish had higher condition (Fig. 11).

Fig. 4. Correlation matrix of energetic and morphometric variables for winter flounder (see Table 3 for variable descriptions and abbreviations). The shape and color of the ellipses indicate the strength and sign of correlations between pairs of variables. Nonsignificant correlations ($P > 0.05$) are indicated with a × symbol. [Colour online.]

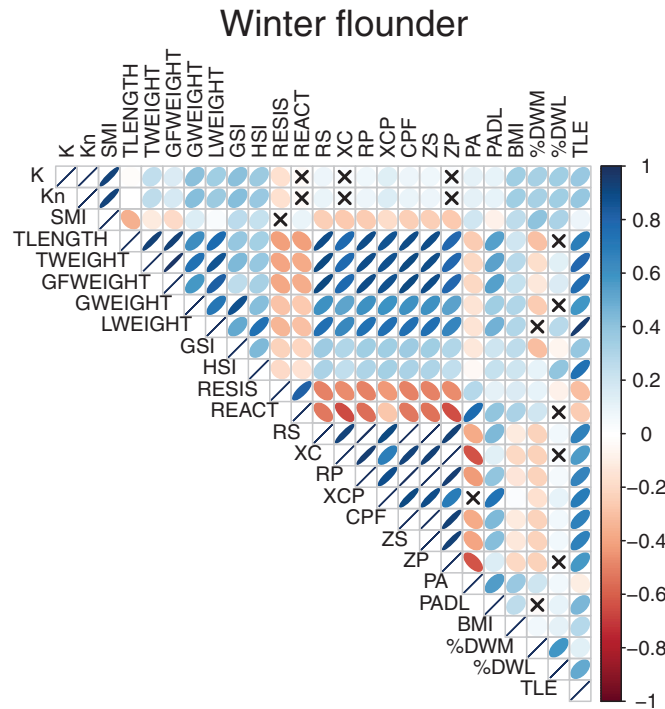


Fig. 5. Correlation matrix of energetic and morphometric variables for yellowtail flounder (see Table 3 for variable descriptions and abbreviations). The shape and color of the ellipses indicate the strength and sign of correlations between pairs of variables. Nonsignificant correlations ($P > 0.05$) are indicated with a × symbol. [Colour online.]

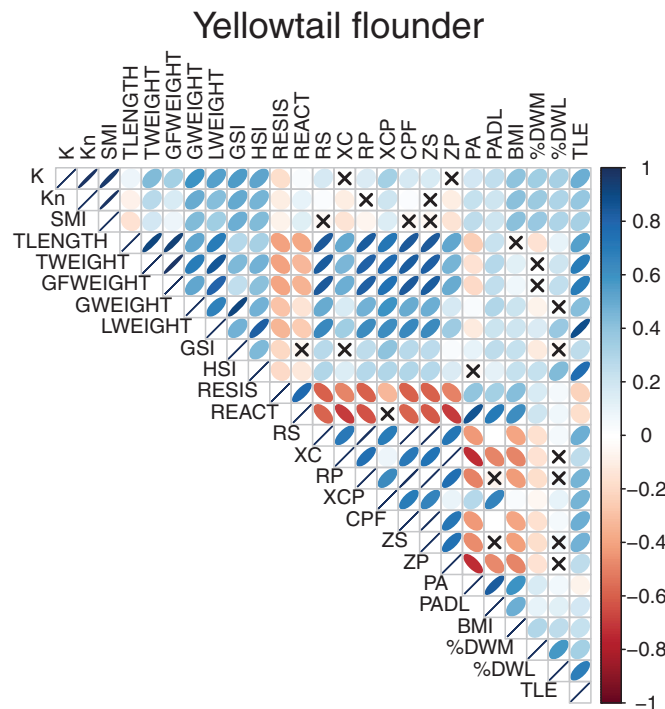
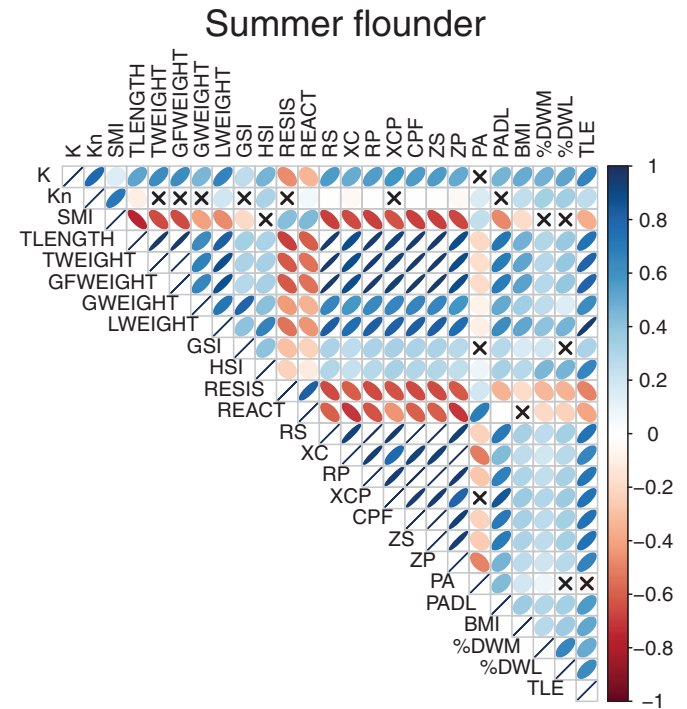


Fig. 6. Correlation matrix of energetic and morphometric variables for summer flounder (see Table 3 for variable descriptions and abbreviations). The shape and color of the ellipses indicate the strength and sign of correlations between pairs of variables. Nonsignificant correlations ($P > 0.05$) are indicated with a × symbol. [Colour online.]



For summer flounder, the first PC explained 57.2% of the observed variation in the data and was again dominated by measures associated with size (Table 6), but also included K. The second PC explained an additional 13.4% of the variance and was related to condition measures K_n , SMI, and K. The third PC explained 7.9% variance and was most strongly associated with PA and REACT. The fourth PC explained only 5.7% of the variation and was positively correlated with GSI in contrast with the other two species. Visualizations of the data by qualitative grouping variables revealed the following: immatures and males were on the left of PC1 (smaller size), and spent fish were slightly lower on PC2 (low condition; Table 6; Fig. 10). Regional (stock) effects were not evaluated for summer flounder.

Discussion

Herein we document large variation in the physiological state of individuals both within and across species. Variation occurred seasonally and across sexes and was due in part to species-specific reproductive and energy allocation patterns. Additional variation was evident across stocks, reflecting large-scale environmental drivers on growth, feeding, and energetics. Morphological indices (based on external and internal characteristics) captured large-scale seasonal differences in condition at the organism level, but were less predictive of biochemical condition at the suborganism level (e.g., energy density of muscle and liver). Some intermediate measures, with respect to ease of collection, such as the percent dry weight of tissue, were good predictors of tissue energy density. Bioelectrical measures generally performed poorly at both the organism and suborganism scales. PCA provided a comprehensive summary of the inter-relationships between various condition measures and how these relationships varied across these three species with very different life histories, providing insights that are applicable to many other species. A more detailed description of

Fig. 7. Relationship between percent dry weight of liver and muscle and energy density for winter, yellowtail, and summer flounder. See Table 5 for regression parameters and test statistics.

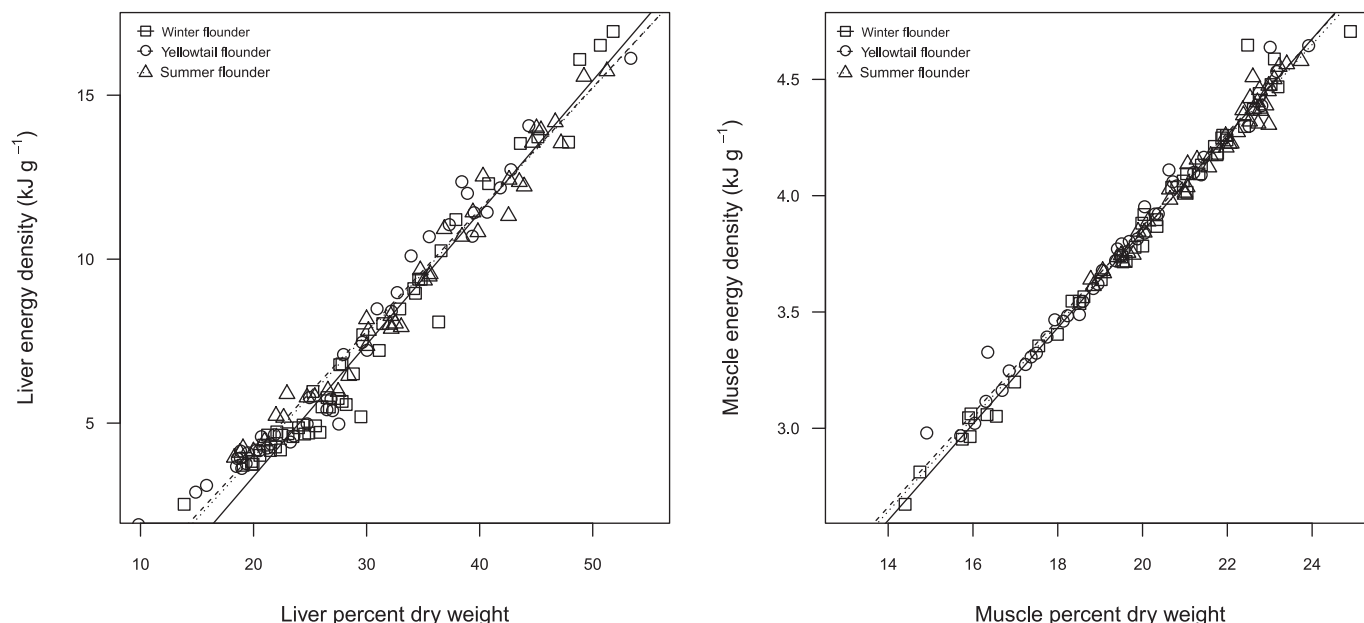


Table 5. Results of linear regressions for tissue energy density (liver or muscle) as a function of tissue percent dry weight (%DWL or %DWM).

	<i>n</i>	Intercept	Slope	Adj. <i>r</i> ²	Prob(> <i>t</i>)
Liver energy density					
Winter flounder	50	-4.67 (0.346)	0.402 (0.0112)	0.963	<0.001
Yellowtail flounder	42	-3.36 (0.341)	0.372 (0.0116)	0.962	<0.001
Summer flounder	43	-3.54 (0.297)	0.375 (0.00816)	0.981	<0.001
Muscle energy density					
Winter flounder	46	-0.294 (0.0728)	0.207 (0.00364)	0.986	<0.001
Yellowtail flounder	40	-0.156 (0.0872)	0.201 (0.00450)	0.981	<0.001
Summer flounder	45	-0.167 (0.107)	0.201 (0.00497)	0.974	<0.001

Note: Estimates of parameters listed with standard error in parentheses.

the variation in fish condition that occurs at various levels (sub-organism, organism, population), the ability of old and new metrics to capture this variation, and therefore the conclusions and advice that can be drawn from them follows.

Seasonal patterns of condition in relation to reproductive strategy and energy allocation

Seasonal cycles in physiological and morphometric variables were evident, even when considering that the summary box plots (Figs. 2, 3) combined sexes and stocks. Variation in spawning season among stocks is well documented for both winter flounder (McBride et al. 2013; McElroy et al. 2013; Press et al. 2014) and yellowtail flounder (McElroy et al. 2016). Aggregating data by month across multiple years, stocks, sexes, and maturity stages likely contributed to the large amount of individual variability in GSI, HSI, and percent dry weight of liver and muscle and dampened the seasonal patterns. Variations among species were also evident, because the reproductive strategies of these three flatfishes follow different processes of energy acquisition, storage, and depletion.

Of the three species studied here, winter flounder underwent the widest seasonal ranges in condition, with significant storage and depletion of energy from muscle tissue. This has been previously documented, with greater variation reported for more northern populations (Plante et al. 2005; Wuenschel et al. 2009). There was limited seasonal variation in liver tissue composition of winter flounder, in contrast with the other two species. Starvation of captive winter flounder was found to result in depletion of a

hypodermal lipid layer along the midline and produced water content levels in the muscle tissue higher (88%–95%) than any observed in wild fish in the current study (Maddock and Burton 1994). The importance of the seasonal timing of feeding and fish condition to reproductive participation has been demonstrated in captive experiments on winter flounder, with poor condition inducing skipped spawning (Burton and Idler 1987; Burton 1994). The characteristics of winter flounder spawning, particularly as a determinate, high fecundity, and total spawner during winter, require this species to utilize its energy reserves (capital) extensively and results in the strong seasonal cycles in condition and %DWM.

Yellowtail flounder also undergo a wide range of condition changes seasonally and showed more individual and seasonal variation in %DWL (and to a lesser degree HSI). Yellowtail flounder also had more individuals at the low end of the %DWM distributions (outliers and lower 25% percentile) over more months. This species was comparable in its patterns with winter flounder, but utilized liver reserves to a greater extent than winter flounder. Dwyer et al. (2003) reported laboratory-reared yellowtail flounder had higher levels of HSI and lipids in the muscle and liver than wild fish, which they attributed to efficient absorption from the diet and rapid deposition in tissues; therefore, the observed seasonal patterns in the current study likely reflect changes in food quality and (or) feeding intensity. In addition, the energy usage of both muscle and liver reserves may reflect its “capital” pattern of reproductive energy usage, but it may be somewhat intermediate

Table 6. Summary of significant variable loadings for the first four principal components (PC) for each species.

PC	Winter flounder (n = 1294)				Yellowtail flounder (n = 1571)				Summer flounder (n = 780)			
	1	2	3	4	1	2	3	4	1	2	3	4
% Explained variance	48.7	17.6	10.8	7.2	42.1	23.8	10.8	7.9	57.2	13.3	7.9	5.7
Correlation												
Variable												
K	0.21	0.85	-0.36	-0.10*	0.38	0.74	-0.42	-0.25	0.65	0.60	-0.27	-0.08*
K _n	0.22	0.85	-0.36	-0.10*	0.23	0.74	-0.49	-0.27	—	0.79	-0.38	—
SMI	-0.14	0.83	-0.43	-0.08*	0.15	0.74	-0.51	-0.28	-0.64	0.61	-0.33	—
TLENGTH	0.92	-0.12	0.25	—	0.86	—	0.39	0.14	0.97	-0.13*	0.10*	-0.08*
TWEIGHT	0.95	0.10*	0.16	—	0.92	0.23	0.20	0.06*	0.98	—	0.08*	-0.09*
GFWEIGHT	0.93	—	0.21	0.12	0.90	0.14	0.23	0.22	0.98	—	0.07*	-0.11*
GWEIGHT	0.72	0.31	—	-0.49	0.64	0.45	0.05*	-0.47	0.72	0.10*	0.15	0.59
LWEIGHT	0.87	0.33	0.09*	—	0.82	0.41	-0.06*	0.17	0.91	0.24	—	0.11*
GSI	0.48	0.36	-0.12	-0.65	0.43	0.47	—	-0.60	0.40	0.12*	0.12*	0.85
HSI	0.46	0.48	-0.08*	0.09*	0.51	0.48	-0.31	0.17	0.43	0.55	—	0.32
RESIS	-0.52	0.11*	0.47	—	-0.61	0.28	0.23	0.25	-0.70	0.09*	0.29	—
REACT	-0.54	0.35	0.70	—	-0.58	0.59	0.41	0.12	-0.62	0.35	0.61	-0.07*
RS	0.97	-0.16	—	0.07*	0.95	-0.21	0.15	—	0.97	-0.14*	—	—
XC	0.89	-0.30	-0.21	0.06*	0.68	-0.53	-0.26	0.05*	0.90	-0.28	-0.20	—
RP	0.97	-0.19	—	0.06*	0.94	-0.26	0.11	—	0.97	-0.17	—	—
XCP	0.89	—	0.31	0.07*	0.69	0.35	0.54	-0.06*	0.94	—	0.24	-0.10*
CPF	0.97	-0.16	—	0.07*	0.95	-0.21	0.15	—	0.97	-0.14*	—	—
ZS	0.97	-0.18	—	0.06*	0.95	-0.23	0.13	—	0.97	-0.16	—	—
ZP	0.90	-0.30	-0.20	0.06*	0.69	-0.53	-0.25	0.05*	0.91	-0.27	-0.18	—
PA	-0.36	0.48	0.66	—	-0.40	0.70	0.47	—	-0.21	0.53	0.72	-0.13*
PADL	0.44	0.29	0.77	0.06*	—	0.68	0.68	—	0.69	0.21	0.59	-0.14*
BMI	0.06*	0.58	0.34	-0.24	-0.19	0.77	0.09*	0.12	0.45	0.40	0.19	-0.12*
%DWM	-0.20	0.47	-0.15	0.69	-0.06*	0.44	-0.37	0.53	0.37	0.55	-0.20	—
%DWL	0.14	0.48	-0.15	0.68	0.19	0.41	-0.31	0.68	0.44	0.53	-0.22	-0.34
TLE	0.79	0.40	0.06*	0.25	0.67	0.43	-0.17	0.47	0.84	0.35	—	—
r²												
Qualitative factors												
REPPHASE	0.21	0.30	0.06	0.17	0.09	0.16	0.06	0.15	0.24	0.02*	—	0.52
SEX	0.07	0.01*	—	0.02	0.11	0.01*	—	0.02	0.07	—	—	0.01*
STOCK	0.09	—	0.08	0.08	0.12	0.27	0.01*	0.04	—	—	—	—

Note: Correlations greater than 0.6 are indicated in bold. The qualitative variables listed are not included in forming the PCs, but how they relate to each PC is tested post hoc. Sample sizes for complete cases used in each analysis is indicated. An asterisk (*) indicates $0.001 < P < 0.05$; all else $P < 0.001$.

compared with the other two species in using both liver and muscle extensively. The species has been shown to exhibit variable levels of down-regulation of fecundity in the period just prior to spawning (McElroy et al. 2016), which may be dependent on available energy reserves (i.e., liver and muscle).

Disease may also play a role in the broad range of %DWL observed among yellowtail flounder. The protozoan parasite *Ichthyophonus* sp. was recently reported in the Georges Bank stock of yellowtail flounder (Huntsberger et al. 2017), and we occasionally observed macroscopic lesions associated with infection (nodules of firm white cysts on the surface of the liver and other peritoneal organs) in all stocks. It is possible the greater variation in %DWL in yellowtail flounder is caused by varying infection intensities, with encysted parasites affecting the percent dry weight and energy content of the liver tissue sample. Interestingly, the relationship between %DWL and liver energy density for yellowtail flounder had a lower r^2 value than the other two species; however, it was still highly correlated ($r^2 = 0.96$; Table 4). Infection by *Ichthyophonus* sp. can spread to other tissues, including muscle and heart, and it is considered to be debilitating and (or) lethal in yellowtail flounder (Huntsberger et al. 2017). Therefore, it is possible that variation in liver condition of yellowtail flounder is reflective of systemic infection in other parts of the body. Although not measured for these samples, increased infection prevalence and intensity may explain some of the low outliers observed for yellowtail flounder in K, K_n, %DWL, and %DWM, which were more common in this species than the other two. This underscores the potential importance of infection and its impacts on growth, reproduction, and condition particularly given the low population status and

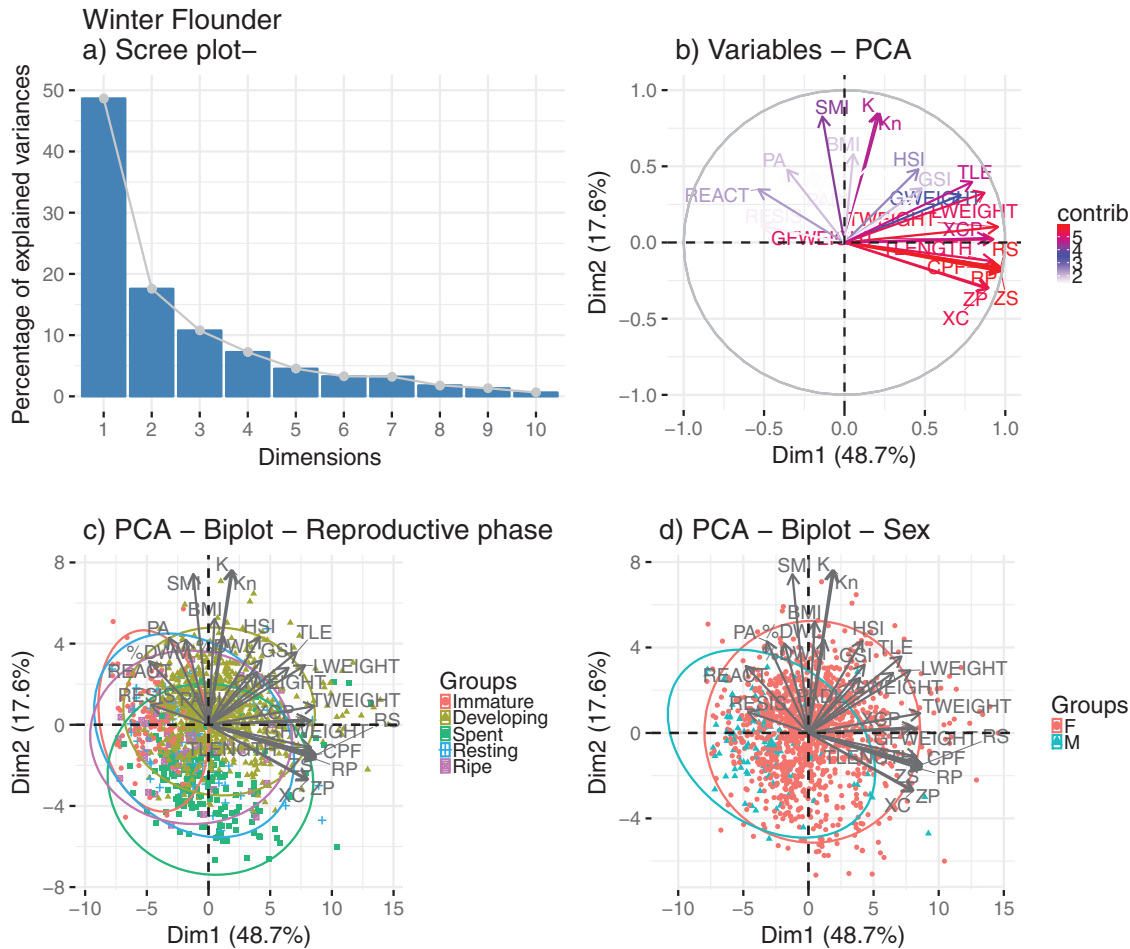
limited recovery of several of the yellowtail flounder stocks (Northeast Fisheries Science Center 2017).

Compared with the other two species, summer flounder exhibited a higher condition with less seasonal variation in %DWM, except for winter flounder in fall. Summer flounder had higher %DWL, which varied over two distinct cycles per year, first peaking in February then again in July–August. Two peaks in liver and muscle condition could be related to the following: (i) summer flounder undergo substantial seasonal migrations, moving offshore and south in winter, and (ii) in addition to the main spawning season occurring in the fall, summer flounder (particularly larger-older individuals) are known to also have a more limited spawning in the spring (evidenced by higher GSI in some individuals during March–April; Fig. 2). Ingressing larvae collected north of Cape Hatteras were mostly fall-spawned, while those south of Cape Hatteras were mostly spring-spawned; however, some overlap in each region was evident (Able et al. 2011). Migration and seasonal prey availability likely plays a larger role influencing the bimodality of liver and muscle energy; by late April and early May summer flounder had moved to inshore waters and condition readily improved in subsequent months. The more limited change in muscle energy in fall and greater use of liver reserves (more reflective of short-term changes) is consistent with the characterization of this species having “income” type reproductive energy allocation pattern.

Interpretation of morphological condition indices – what are they measuring?

The overall seasonal patterns provided contrast in multiple metrics at the organism and suborganism scales, and by including

Fig. 8. Summary PCA plots for winter flounder: (a) scree plot, (b) variables biplot showing contribution from each variable, (c) individuals plotted by reproductive phase, (d) individuals plotted by sex. See Table 3 for variable descriptions and abbreviations.



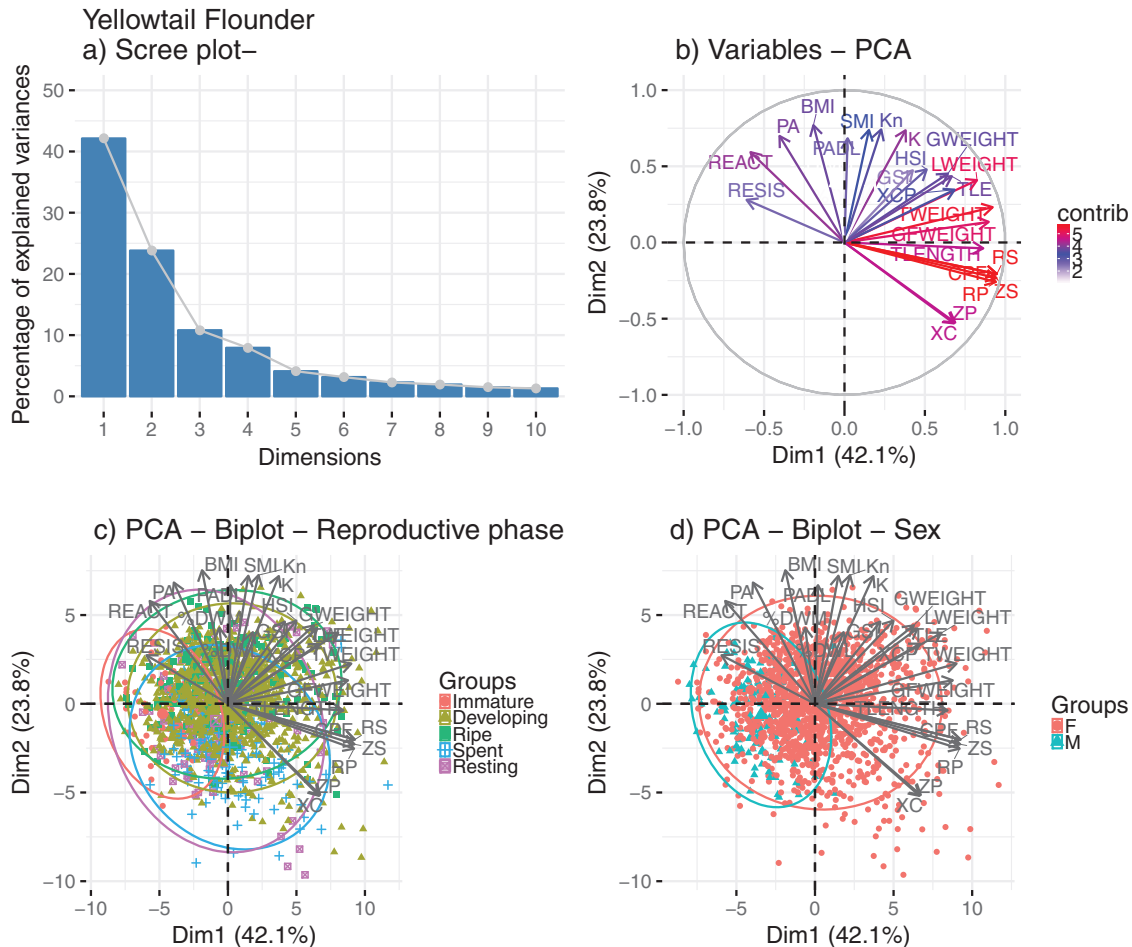
all of these variables in the same multivariate analysis, we were able to summarize relationships between them. PCA variables represented by vectors that point in the same direction have a similar response profile and convey similar meaning (i.e., may be redundant, whereby simpler measures are suitable proxies for more difficult ones). Condition indices derived from morphological (external — e.g., length, weight; internal — e.g., liver or gonad weight) properties of fishes are widely used owing their ease of application; however, the value of such indices to serve as reliable proxies for biochemical condition is often equivocal (Davidson and Marshall 2010; McPherson et al. 2011; Brosset et al. 2015; Sardenne et al. 2016; Morgan et al. 2018). For the three species investigated here, external morphological condition indices provided marginal direct estimates of measured biochemical condition (muscle and liver energy density). For both winter and yellowtail flounder, the PCA loadings for morphological (K , K_n , SMI) and biochemical (%DWM, %DWL) variables were both aligned with PC2, supporting use of these metrics to infer biochemical condition. One drawback of using K_n for monitoring studies is the need to recalculate length–weight regression to obtain the predicted weight, as new observations are added. In the present study, we partially avoided this; the “new” data we have added throughout the study was not seasonally balanced so we opted to avoid biasing of new length–weight equations and continued to use those determined during the first years of seasonally balanced samples. This modification has potential to shift the center of observation from 1, but we feel it still provides the interpretability we are after (i.e., fish greater than 1 are “above” average). The

biochemical variables were less strongly associated with PC2 for summer flounder, cautioning the use of K , K_n , or SMI to infer biochemical condition. Contrast in external morphological condition indices (e.g., K_n) has been identified as a prerequisite for significant correlations with biochemical indices. Lambert and Dutil (1997) found significant correlations for cod (*Gadus morhua*, K_n range 0.45–1.2), while correlations for tropical tunas were not significant (bigeye (*Thunnus pelamis*), skipjack (*Katsuwonis pelamis*), and yellowfin tuna (*Thunnus albacares*); K_n range 0.8–1.2) (Sardenne et al. 2016). K_n of winter and yellowtail flounder ranged widely across seasons (0.6–1.4; Fig. 3), and sampling over multiple years captured variation in condition to reasonably test relationships. Even with this contrast, although the morphometric indices aligned with PC2 (i.e., condition), they performed poorly as single predictors of biochemical condition. K_n of summer flounder was more stable, but still exhibited seasonal variation (K_n range 0.7–1.3) that was weakly correlated to biochemical condition. The biochemical condition indices evaluated here (muscle and liver energy density) may not capture the “overall” condition given they are derived from specific body components; however, muscle and liver are major sites of energy storage in fishes.

Relationship between percent dry weight of muscle and liver and energy density

As in other studies, we found strong relationships between the percent dry weight and energy density of fish tissue, in this case for liver and muscle. Percent dry weight is well documented as a good predictor of energy density of whole fish (Hartman and

Fig. 9. Summary PCA plots for yellowtail flounder: (a) scree plot, (b) variables biplot showing contribution from each variable, (c) individuals plotted by reproductive phase, (d) individuals plotted by sex. See Table 3 for variable descriptions and abbreviations.



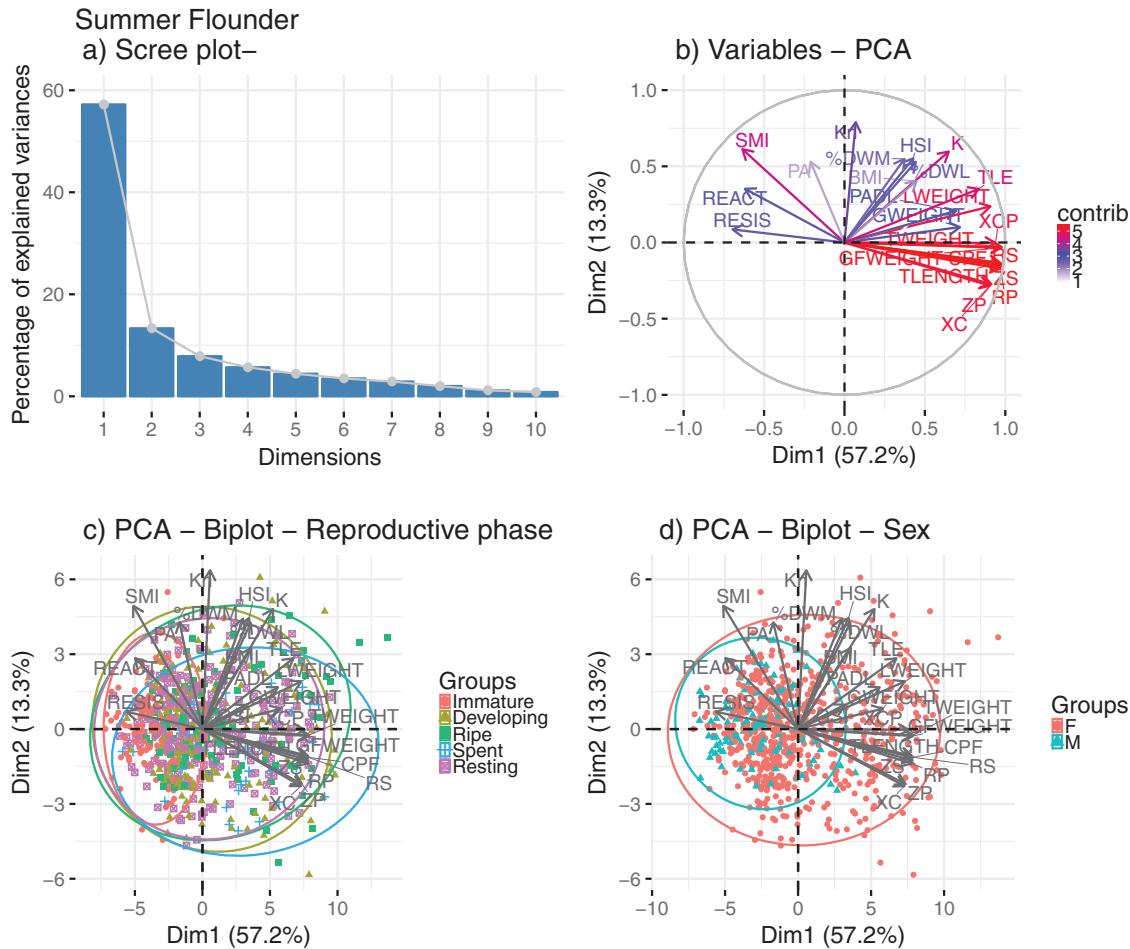
Brandt 1995; Schreckenbach et al. 2001; Glover et al. 2010; Johnson et al. 2017) and fish tissue (Morley et al. 2012; Wuenschel et al. 2013b). While this relationship can vary across taxonomic groups and ontogenetic stages (Hartman and Brandt 1995; Wuenschel et al. 2006; Schloesser and Fabrizio 2015, 2017), the three flatfishes evaluated here were remarkably similar in energy density at a given percent dry weight. One notable difference was the range in percent dry weight of muscle observed for each species, where summer flounder was found to maintain higher muscle energy (i.e., not deplete it) throughout the year, especially following spawning. This finding is consistent with the assumption that summer flounder is largely an income breeder, allocating ingested energy directly to reproduction during a protracted spawning period. The lipid composition of adult summer flounder prey (fish and squid) has a role in maintaining higher energy levels throughout the year compared with the other two flatfishes. Although the three species also had similar and strong relations in %DWL and the energy density of the liver, a slight curvilinear effect was apparent. This implies less lipid (energy) is stored until a threshold level of percent dry weight is reached, possibly reflecting changes in structural components in the liver. Liver glycogen and lipid levels of cultured yellowtail flounder were higher than that of wild fish, and females had more lipids in hepatocytes than males (Fahraeus-Van Ree and Spurrell 2003). A much more pronounced biphasic relation has been shown in the muscle of bluefish (*Pomatomus saltatrix*) (Morley et al. 2012).

Evaluation of alternatives to traditional morphometric condition indices

Given the limited utility of traditional morphological condition indices to capture changes in biochemical condition of fishes, various methods have recently been proposed, including SMI (Peig and Green 2009) and bioelectrical impedance analysis (BIA; Cox and Hartman 2005; Cox et al. 2011; Hartman et al. 2015). As in other applications (Peig and Green 2010; Maceda-Veiga et al. 2014), patterns in SMI were different than other condition metrics based on external morphology (e.g., K , K_n). In winter and yellowtail flounder, SMI was poorly correlated with all measures evaluated. The PCAs for these two species indicated low contribution of SMI to explaining variation in the data. However, in summer flounder, SMI was negatively correlated with size (TLENGTH and other measures strongly related to TLENGTH: GWEIGHT, LWEIGHT). The contribution of SMI to the summer flounder PCA was greater and significant, but was not strongly aligned with either PC1 or PC2, complicating interpretation.

In all three species, K and K_n were significantly positively correlated with percent dry weight of muscle and liver, but the relationships were generally weak ($r = 0.4$ to 0.5), while SMI had very weak or insignificant correlations with these two measures. K , which has been shown to have significant length bias, was not strongly correlated with length in winter or yellowtail flounder, but was more strongly correlated for summer flounder (where a larger range in sizes was analyzed). This may be due in part to sources of material analyzed (predominately commercial gears)

Fig. 10. Summary PCA plots for summer flounder: (a) scree plot, (b) variables biplot showing contribution from each variable, (c) individuals plotted by reproductive phase, (d) individuals plotted by sex. See Table 3 for variable descriptions and abbreviations.



that collected a more limited size distribution. In addition, given the current low levels of the yellowtail and winter flounder stocks (Northeast Fisheries Science Center 2017), larger sizes and older ages were not encountered and analyzed, which may have reduced our ability to detect differences due to size. For each species, the largest size analyzed herein was much lower than maximum sizes reported (Tables 1, 2; winter flounder 548 versus 640 mm; yellowtail flounder 503 versus 640 mm; summer flounder 736 versus 940 mm). Because of potential for size bias in K, especially for fishes with dimorphic growth (including the three species studied here), comparisons should be limited to samples of similar size and sex.

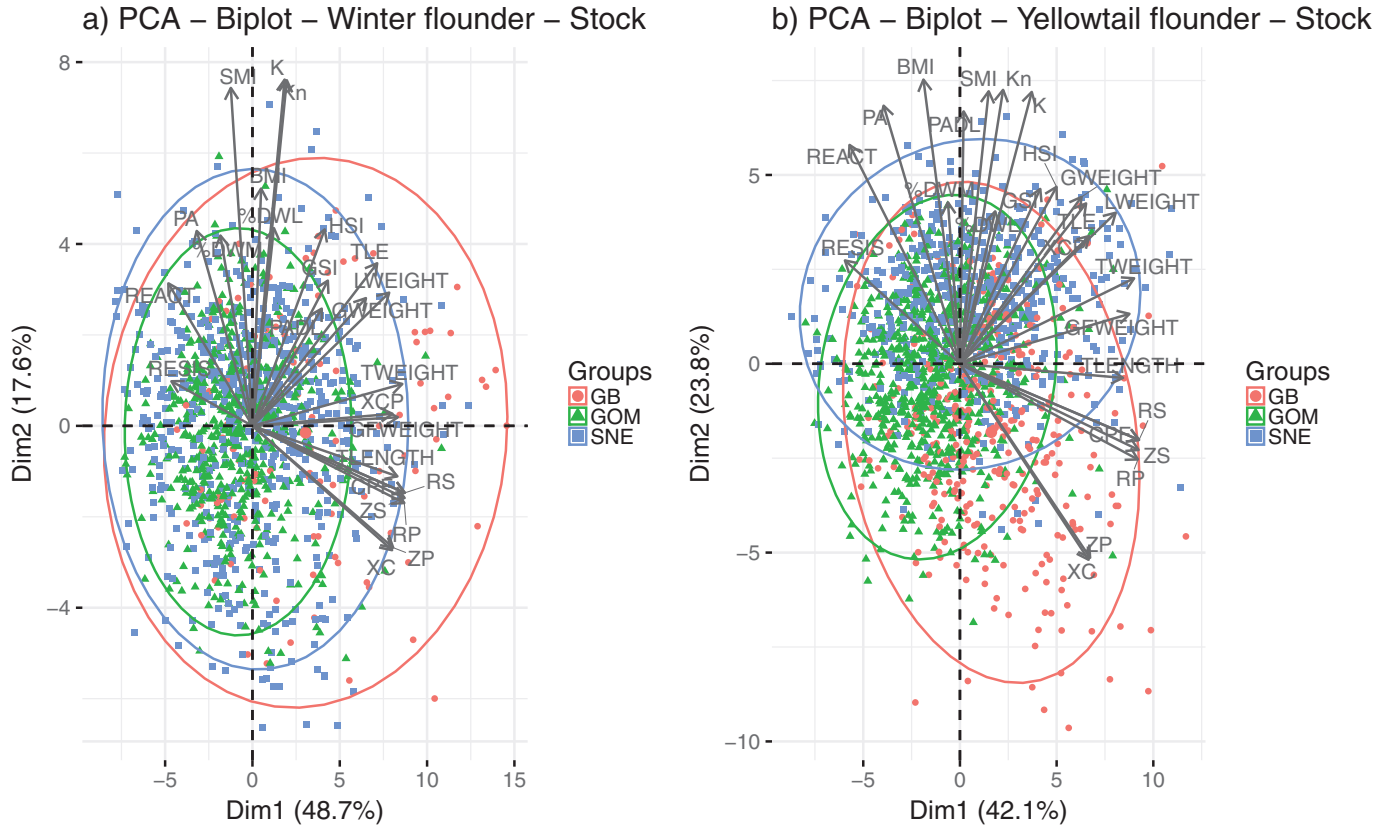
Bioelectrical variables were poorly correlated with morphological condition metrics (K , K_m , SMI) in winter and yellowtail flounder. Phase angle, calculated from BIA measured resistance and reactance, has been positively related to body condition (Cox and Heintz 2009), weakly related to future gonadal investment (Wuenschel et al. 2013b), and may be sensitive to medium-term metabolic states (e.g., integrated over weeks).

The BIA for the three flounders studied here was collected in a standardized manner using an electrode holder assembly to minimize variation due to electrode stability, placement, and measurement of electrode distance. In contrast with other BIA studies, the data reported here were obtained from dead fish that were iced for varying periods, which has been shown to affect BIA readings (Cox and Heintz 2009; Cox et al. 2011). The resistance of coho salmon (*Oncorhynchus kisutch*) remained stable for more than 24 h after death, while the reactance began to change within a few

hours (Cox et al. 2011); therefore, the BIA measures reported here from fish samples (~12 to 24 h) after death likely do not accurately reflect values for those same fish when alive, potentially adding “noise” and obscuring more positive relations. Knowing this potential limitation, we hoped a strong “signal” given the wide range of condition would overcome the variation due to time after death, but this did not occur. Other aspects of sample quality were not affected in a similar manner, including the percent dry weight analyses of liver and muscle and gonad histology (see Press et al. 2014); BIA seems especially sensitive to freshness of the sample.

Although BIA is reflective of somatic muscle energy of juvenile salmonids (Hanson et al. 2010), relations between muscle energy (%DWM) and BIA measures were weak in the flatfishes examined here. Hartman et al. (2015) recently reviewed application of BIA in fish studies, noting successes and failures, with recommendations for obtaining accurate estimates of body composition in fish. One well-documented potential limitation of BIA measures is the need to consider temperature effects on measured resistance and reactance (Hartman et al 2011; Hafis and Hartman 2015). In the present study all measurements were obtained in the lab, at a consistent temperature, so variation in temperature at time of measurement is not considered to be a significant source of error in our results. BIA measures have been shown to be sensitive to the freezing process (Cox 2015) and used as an indicator of previously frozen samples (Vidacek et al. 2008, 2012), which may have occurred for some of our samples while on deck during winter months and then transported on ice. In addition, our laboratory protocol included testing resistance and reactance through the needle elec-

Fig. 11. PCA biplots showing stock effects for (a) winter flounder and (b) yellowtail flounder.



trodes for a known resistor wired with electrical clips directly connected to the needle electrodes. This ensured the unit and electrodes provided a solid connection prior to each sampling session, and it is considered to be an improvement over other protocols that tested standard resistors but not through the entire needle electrode assembly. Studies have also noted experience as a potential source of error (Cox et al. 2011), which was minimized in our study where measures were made by few (<5) individuals, with substantial training, oversight, and overlap.

Bioelectrical variables were highly correlated with each other, and also with many size-related measures, which can be problematic. Given the electrical properties were all calculated using the measured resistance and (or) reactance, it is not surprising that many of these variables were strongly correlated with each other (see Dibble et al. 2017). Despite the inherent co-dependencies between these calculated BIA variables, studies have often included many likely redundant variables in regression models developed to predict body composition from BIA measures (e.g., Hafs and Hartman 2011, 2014; Hartman et al. 2011; Stolarski et al. 2014). In practice, when such co-dependencies exist in the underlying data, the parameter estimates and significance of given variables are unstable and change with the addition of “new” data, limiting their predictive value. This may explain why different studies (even on similar or related species) have included different sets of BIA variables in predictive models (Cox and Hartman 2005; Rasmussen et al. 2012; Hafs and Hartman 2014). In general, the results of the PCA for each species indicated several BIA variables were strongly related to size (i.e., PC1) while others were more related to condition (i.e., PC2), but in each case BIA variables explained less or similar amounts of variation (shorter vectors in biplots and lower variable loadings; Table 5) than either traditional size metrics (along PC1) or condition metrics (along PC2). The energetic variables analyzed here were percentage-based (i.e., percent dry weight of muscle and liver tissue) and not whole body

estimates (e.g., total body water, lipid, energy), which have generally been more successfully related to BIA (Hartman et al. 2015). As in other studies demonstrating low success of BIA to predict percentage-based metrics (Pothoven et al. 2008; Caldarone et al. 2012), our results suggest BIA is of limited value to estimate percent dry weight and energy content of muscle and liver in these flatfish.

Available metrics to assess fish condition actually quantify different aspects of condition; therefore, the optimal metric for any study will depend on the specific goals or objectives (e.g., single species energy content and reproductive potential, or multispecies ecosystem indicators). The life history (particularly reproductive strategy) and energy allocation (e.g., where are lipids stored) mode of a study species can also influence the utility of certain metrics, and in some cases several may need to be evaluated to identify the most appropriate. Fulton’s K and relative condition K_n performed similarly; however, K_n has advantages of being centered on 1 and is thus more easily interpreted. SMI was slightly more complicated to calculate (as compared with K and K_n) and provided little additional explanatory power to these simpler metrics in the correlation plots and the PCAs. In addition, while SMI tracked condition of winter and yellowtail flounders, its values for summer flounder confounded fish size and condition. Although some condition metrics offer simplicity, they may not reflect the same aspects of condition across species or life stages. Therefore, interpretation of simple morphometric indices is limited without validation to more specific indices (e.g., fat or energy content). BIA measures were not indicative of condition. Gonadosomatic or hepatosomatic indices are appropriate metrics of seasonal condition around reproductive seasons within a single stock, but the scale tends to vary by species, complicating multispecies comparisons. The percent dry weight is a good proxy for whole body or tissue-specific energy density and is an effective intermediate (in terms of logistics and cost) measure that can be tailored to species-

specific energy allocation. K_n avoids potential size biases and performed the best consistently across the three very different species examined, implying suitability for single and multispecies and ecosystem analyses. Consideration of multiple metrics, as done here, enables more robust interpretation of condition and reveals potential deficiencies in specific metrics.

Acknowledgements

This study was funded by the National Oceanic and Atmospheric Administration (NOAA), NEFSC, and CRB. Most samples were provided by the CRB's Study Fleet. We appreciate the work of all the Study Fleet staff (especially J. Hoey, J. Moser, M. Ball, D. St. Amand) and all the participating fishermen. We thank all the staff members of these various programs: the officers and crew of the NOAA research vessel *H.B. Bigelow* and the scientific staff of the Ecosystems Surveys Branch of the NEFSC; J. King, M. Camisa, V. Manfredi, and M. Symanski of the MADMF; J. Collie and A. Malek of URI. Finally, we thank E. Tholke, Y. Press, M. Winton, and J. Dayton (NEFSC) for the contributions to data collection and protocol development; A. Aruda for proximate composition analysis of tissue samples; and Phil E. Speshel for inspiration; L. Smith, S. Gaichas, C. Legault, and anonymous reviewers for helpful comments on the manuscript. Mention of any products is for descriptive purposes and does not indicate endorsement by NOAA Fisheries.

References

- Able, K.W., Sullivan, M.C., Hare, J.A., Bath-Martin, G., Taylor, J.C., and Hagan, R. 2011. Larval abundance of summer flounder (*Paralichthys dentatus*) as a measure of recruitment and stock status. *Fish. Bull.* **109**(1): 68–78.
- Blackwell, B.G., Brown, M.L., and Willis, D.W. 2000. Relative weight (Wr) status and current use in fisheries assessment and management. *Rev. Fish. Sci.* **8**: 1–44. doi:10.1080/10641260091129161.
- Brosset, P., Fromentin, J.M., Menard, F., Pernet, F., Bourdeix, J.H., Bigot, J.L., Van Beveren, E., Roda, M.A.P., Choy, S., and Sarau, C. 2015. Measurement and analysis of small pelagic fish condition: a suitable method for rapid evaluation in the field. *J. Exp. Mar. Biol. Ecol.* **462**: 90–97. doi:10.1016/j.jembe.2014.10.016.
- Brosset, P., Fromentin, J.M., Van Beveren, E., Lloret, J., Marques, V., Basilone, G., Bonanno, A., Carpi, P., Donato, F., Kec, V.C., De Felice, A., Ferreri, R., Gasperevic, D., Giraldez, A., Gucu, A., Iglesias, M., Leonori, I., Palomera, I., Somarakis, S., Ticina, V., Torres, P., Ventero, A., Zorica, B., Menard, F., and Sarau, C. 2017. Spatio-temporal patterns and environmental controls of small pelagic fish body condition from contrasted Mediterranean areas. *Prog. Oceanogr.* **151**: 149–162. doi:10.1016/j.pcean.2016.12.002.
- Burnett, J., O'Brien, L., Mayo, R.K., Darde, J.A., and Bohan, M. 1989. Finfish maturity sampling and classification schemes used during Northeast Fisheries Center bottom trawl surveys, 1963–1989. NOAA Tech. Memo. NMFS-F/NEC-76.
- Burton, M.P.M. 1994. A critical period for nutritional control of early gametogenesis in female winter flounder, *Pleuronectes americanus* (Pisces: Teleostei). *J. Zool. Lond.* **233**(3): 405–415. doi:10.1111/j.1469-7998.1994.tb05273.x.
- Burton, M.P., and Idler, D.R. 1987. An experimental investigation of the non-reproductive, post-mature state in winter flounder. *J. Fish Biol.* **30**(6): 643–650. doi:10.1111/j.1095-8649.1987.tb05793.x.
- Cade, B.S., Terrell, J.W., and Porath, M.T. 2008. Estimating fish body condition with quantile regression. *N. Am. J. Fish. Manage.* **28**(2): 349–359. doi:10.1577/M07-048.1.
- Caldarone, E.M., MacLean, S.A., and Sharack, B. 2012. Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo solar*) to food availability. *Fish. Bull.* **110**(2): 257–270.
- Camizuli, E., Monna, F., Scheifler, R., Amiotte-Suchet, P., Losno, R., Beis, P., Bohard, B., Chateau, C., and Alibert, P. 2014. Impact of trace metals from past mining on the aquatic ecosystem: a multi-proxy approach in the Morvan (France). *Environ. Res.* **134**: 410–419. doi:10.1016/j.envres.2014.07.008. PMID: 2525284.
- Choi, J.S., Frank, K.T., Leggett, W.C., and Drinkwater, K. 2004. Transition to an alternate state in a continental shelf ecosystem. *Can. J. Fish. Aquat. Sci.* **61**(4): 505–510. doi:10.1139/f04-079.
- Cone, R.S. 1989. The need to reconsider the use of condition indices in fishery science. *Trans. Am. Fish. Soc.* **118**(5): 510–514. doi:10.1577/1548-8659(1989)118<0511:TNTRTU>2.3.CO;2.
- Cox, M.K. 2015. Bioelectrical impedance analysis measures of body composition and condition, and its sensitivity to the freezing process. *J. Aquat. Food Prod. Technol.* **24**(4): 368–377. doi:10.1080/10498850.2013.777863.
- Cox, M.K., and Hartman, K.J. 2005. Nonlethal estimation of proximate composition in fish. *Can. J. Fish. Aquat. Sci.* **62**(2): 269–275. doi:10.1139/f04-180.
- Cox, M.K., and Heintz, R. 2009. Electrical phase angle as a new method to measure fish condition. *Fish. Bull.* **107**(4): 477–487.
- Cox, M.K., Heintz, R., and Hartman, K.J. 2011. Measurements of resistance and reactance in fish with the use of bioelectrical impedance analysis: sources of error. *Fish. Bull.* **109**(1): 34–47.
- Crossin, G.T., and Hinch, S.G. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific Salmon. *Trans. Am. Fish. Soc.* **134**(1): 184–191. doi:10.1577/FT04-076.1.
- Davidson, D., and Marshall, C.T. 2010. Are morphometric indices accurate indicators of stored energy in herring *Clupea harengus*? *J. Fish Biol.* **76**(4): 913–929. doi:10.1111/j.1095-8649.2010.02546.x.
- DeCelles, G.R., and Cadrin, S.X. 2011. An interdisciplinary assessment of winter flounder (*Pseudopleuronectes americanus*) stock structure. *J. Northw. Atl. Fish. Sci.* **43**: 103–120. doi:10.2960/j.v43.m673.
- Dibble, K.L., Yard, M.D., Ward, D.L., and Yackulic, C.B. 2017. Does bioelectrical impedance analysis accurately estimate the physiological condition of threatened and endangered desert fish species? *Trans. Am. Fish. Soc.* **146**: 888–902. doi:10.1080/00028487.2017.1302993.
- Dwyer, K.S., Parrish, C.C., and Brown, J.A. 2003. Lipid composition of yellowtail flounder (*Limanda ferruginea*) in relation to dietary lipid intake. *Mar. Biol.* **143**(4): 659–667. doi:10.1007/s00227-003-1101-0.
- Fahraeus-Van Ree, G.E., and Spurrell, D.R. 2003. Structure of and energy reserves in the liver of wild and cultured yellowtail flounder, *Limanda ferruginea*. *Mar. Biol.* **143**(2): 257–265. doi:10.1007/s00227-003-1083-y.
- Fairchild, E.A., Siceloff, L., Howell, W.H., Hoffman, B., and Armstrong, M.P. 2013. Coastal spawning by winter flounder and a reassessment of Essential Fish Habitat in the Gulf of Maine. *Fish. Res.* **141**: 118–129. doi:10.1016/j.fishres.2012.05.007.
- Fitzhugh, G.R., Wuenschel, M.J., and McBride, R.S. 2010. Evaluation of bioelectrical impedance analysis (BIA) to measure condition and energy allocated to reproduction in marine fishes. *J. Phys. Conf. Ser.* **224**(1): 012137. doi:10.1088/1742-6596/224/1/012137.
- Garner, S.B., van der Ham, J.L., Boswell, K.M., and Cowan, J.H. 2012. Use of bioelectric impedance analysis to assess total-body condition and predict energy density in juvenile Atlantic croaker. *Trans. Am. Fish. Soc.* **141**(4): 1117–1125. doi:10.1080/00028487.2012.675894.
- Glover, D.C., DeVries, D.R., Wright, R.A., and Davis, D.A. 2010. Sample preparation techniques for determination of fish energy density via bomb calorimetry: an evaluation using largemouth bass. *Trans. Am. Fish. Soc.* **139**(3): 671–675. doi:10.1577/T09-110.1.
- Hafs, A.W., and Hartman, K.J. 2011. Influence of electrode type and location upon bioelectrical impedance analysis measurements of brook trout. *Trans. Am. Fish. Soc.* **140**(5): 1290–1297. doi:10.1080/00028487.2011.620482.
- Hafs, A.W., and Hartman, K.J. 2014. Developing bioelectrical impedance analysis methods for age-0 brook trout. *Fish. Manage. Ecol.* **21**(5): 366–373. doi:10.1111/fme.12086.
- Hafs, A.W., and Hartman, K.J. 2015. Development of temperature correction equations for bioelectrical impedance analysis models for brook trout *Salvelinus fontinalis*. *J. Fish Biol.* **86**(1): 304–316. doi:10.1111/jfb.12583. PMID: 25425157.
- Hanson, K.C., Ostrand, K.G., Gannam, A.L., and Ostrand, S.L. 2010. Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. *Trans. Am. Fish. Soc.* **139**(6): 1733–1741. doi:10.1577/T10-014.1.
- Hartman, K.J., and Brandt, S.B. 1995. Estimating energy density of fish. *Trans. Am. Fish. Soc.* **124**(3): 347–355. doi:10.1577/1548-8659(1995)124<0347:EEDOF>2.3.CO;2.
- Hartman, K.J., Phelan, B.A., and Rosendale, J.E. 2011. Temperature effects on bioelectrical impedance analysis (BIA) used to estimate dry weight as a condition proxy in coastal bluefish. *Mar. Coast. Fish.* **3**: 307–316. doi:10.1080/19425120.2011.603961.
- Hartman, K.J., Margraf, F.J., Hafs, A.W., and Cox, M.K. 2015. Bioelectrical impedance analysis: a new tool for assessing fish condition. *Fisheries*, **40**(12): 590–600. doi:10.1080/03632415.2015.1106943.
- Hayes, J.P., and Shonkwiler, J.S. 2001. Morphological indicators of body condition: useful or wishful thinking? *In* Body composition analysis of animals: a handbook of non-destructive methods. Edited by J.R. Speakman. Cambridge University Press, Cambridge, UK. pp. 8–38.
- Henken, A.M., Lucas, H., Tijssen, P.A.T., and Machiels, M.A.M. 1986. A comparison between methods used to determine the energy content of feed, fish and feces samples. *Aquaculture*, **58**(3–4): 195–201. doi:10.1016/0044-8486(86)90085-2.
- Huntsberger, C.J., Hamlin, J.R., Smolowitz, R.J., and Smolowitz, R.M. 2017. Prevalence and description of *Ichthyophonus* sp. in yellowtail flounder (*Limanda ferruginea*) from a seasonal survey on Georges Bank. *Fish. Res.* **194**: 60–67. doi:10.1016/j.fishres.2017.05.012.
- Johnson, B.M., Pate, W.M., and Hansen, A.G. 2017. Energy density and dry matter content in fish: new observations and an evaluation of some empirical models. *Trans. Am. Fish. Soc.* **146**(6): 1262–1278. doi:10.1080/00028487.2017.1366392.
- Kassambara, A., and Mundt, F. 2016. Factoextra: Extract and visualize the results of multivariate data analyses [online]. Available from <https://CRAN.R-project.org/package=factoextra>; r package version 1.0.3, 2016.
- Klefoth, T., Skov, C., Aarestrup, K., and Arlinghaus, R. 2013. Reliability of non-lethal assessment methods of body composition and energetic status exem-

- plified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*). Fish. Res. **146**: 18–26. doi:10.1016/j.fishres.2013.03.010.
- Klein-MacPhee, G. 2002a. Righteye flounders, Family Pleuronectidae. In Bigelow and Schroeder's fishes of the Gulf of Maine. Edited by B.B. Collette and G. Klein-MacPhee. Smithsonian Institution. pp. 560–587.
- Klein-MacPhee, G. 2002b. Sand flounders, Family Paralichthyidae. In Bigelow and Schroeder's fishes of the Gulf of Maine. Edited by B.B. Collette and G. Klein-MacPhee. Smithsonian Institution. pp. 551–560.
- Lambert, Y., and Dutil, J.-D. 1997. Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Can. J. Fish. Aquat. Sci. **54**(10): 2388–2400. doi:10.1139/f97-145.
- Larsen, D.A., Middleton, M.A., Dickey, J.T., Gerstenberger, R.S., Brun, C.V., and Swanson, P. 2017. Use of morphological and physiological indices to characterize life history diversity in juvenile hatchery winter-run steelhead. Trans. Am. Fish. Soc. **146**(4): 663–679. doi:10.1080/00028487.2017.1296492.
- Le Cren, E.D. 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. **20**: 201–219. doi:10.2307/1540.
- Le, S., Josse, J., and Husson, F. 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. **25**(1): 1–18. doi:10.18637/jss.v025.i01.
- Link, J.S., and Burnett, J. 2001. The relationship between stomach contents and maturity state for major northwest Atlantic fishes: new paradigms? J. Fish Biol. **59**(4): 783–794. doi:10.1111/j.1095-8649.2001.tb00149.x.
- Link, J.S., Bolles, K., and Milliken, C.G. 2002. The feeding ecology of flatfish in the northwest Atlantic. J. Northwest Atl. Fish. Sci. **30**: 1–17. doi:10.2960/j.v30.a1.
- Lleonart, J., Salat, J., and Torres, G.J. 2000. Removing allometric effects of body size in morphological analysis. J. Theor. Biol. **205**(1): 85–93. doi:10.1006/jtbi.2000.2043. PMID:10860702.
- Love, R.M. 1980. The chemical biology of fishes: volume 2, advances 1968–1977: with a supplementary key to the chemical literature. Academic Press, London, New York.
- MacCracken, J.G., and Stebbings, J.L. 2012. Test of a body condition index with amphibians. J. Herpetol. **46**(3): 346–350. doi:10.1670/10-292.
- Maceda-Veiga, A., Green, A.J., and De Sostoa, A. 2014. Scaled body-mass index shows how habitat quality influences the condition of four fish taxa in northeastern Spain and provides a novel indicator of ecosystem health. Freshw. Biol. **59**(6): 1145–1160. doi:10.1111/fwb.12336.
- Maddock, D.M., and Burton, M.P.M. 1994. Some effects of starvation on the lipid and skeletal muscle layers of the winter flounder, *Pleuronectes americanus*. Can. J. Zool. **72**(9): 1672–1679. doi:10.1139/z94-223.
- Marshall, C.T., Yaragina, N.A., Lambert, Y., and Kjesbu, O.S. 1999. Total lipid energy as a proxy for total egg production by fish stocks. Nature. **402**: 288–290. doi:10.1038/46272.
- Marshall, C.T., O'Brien, L., Tomkiewicz, J., Koster, F.W., Kraus, G., Martensdottir, G., Morgan, M.J., Saborido-Rey, F., Blanchard, J.L., Secor, D.H., Wright, P.J., Mukhina, N.V., and Bjornsson, H. 2003. Developing alternative indices of reproductive potential for use in fisheries management: case studies for stocks spanning an information gradient. J. Northwest Atl. Fish. Sci. **33**: 161–190. doi:10.2960/j.v33.a8.
- Marshall, C.T., Needle, C.L., Yaragina, N.A., Ajiad, A.M., and Gusev, E. 2004. Deriving condition indices from standard fisheries databases and evaluating their sensitivity to variation in stored energy reserves. Can. J. Fish. Aquat. Sci. **61**(10): 1900–1917. doi:10.1139/f04-128.
- Marshall, C.T., Needle, C.L., Thorsen, A., Kjesbu, O.S., and Yaragina, N.A. 2006. Systematic bias in estimates of reproductive potential of an Atlantic cod (*Gadus morhua*) stock: implications for stock–recruit theory and management. Can. J. Fish. Aquat. Sci. **63**(5): 980–994. doi:10.1139/f05-270.
- Masse, J., Sanchez, F., Delaunay, D., Robert, J.M., and Petitgas, P. 2016. A partnership between science and industry for a monitoring of anchovy & sardine in the Bay of Biscay: when fishermen are actors of science. Fish. Res. **178**: 26–38. doi:10.1016/j.fishres.2015.11.018.
- McBride, R.S., Wuenschel, M.J., Nitschke, P., Thornton, G., and King, J.R. 2013. Latitudinal and stock-specific variation in size- and age-at-maturity of female winter flounder, *Pseudopleuronectes americanus*, as determined with gonad histology. J. Sea Res. **75**: 41–51. doi:10.1016/j.seares.2012.04.005.
- McBride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A., Wuenschel, M.J., Alonso-Fernandez, A., and Basilone, G. 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish Fish. **16**(1): 23–57. doi:10.1111/faf.12043.
- McElroy, W.D., Wuenschel, M.J., Press, Y.K., Towle, E.K., and McBride, R.S. 2013. Differences in female individual reproductive potential among three stocks of winter flounder, *Pseudopleuronectes americanus*. J. Sea Res. **75**: 52–61. doi:10.1016/j.seares.2012.05.018.
- McElroy, W.D., Wuenschel, M.J., Towle, E.K., and McBride, R.S. 2016. Spatial and annual variation in fecundity and oocyte atresia of yellowtail flounder, *Limanda ferruginea*, in U.S. waters. J. Sea Res. **107**(1): 76–89. doi:10.1016/j.seares.2015.06.015.
- McPherson, L.R., Slotte, A., Kvamme, C., Meier, S., and Marshall, C.T. 2011. Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). ICES J. Mar. Sci. **68**(1): 52–60. doi:10.1093/icesjms/fsq148.
- Merson, R.R., Casey, C.S., Martinez, C., Soffientino, B., Chandlee, M., and Specker, J.L. 2000. Oocyte development in summer flounder: seasonal changes and steroid correlates. J. Fish Biol. **57**(1): 182–196. doi:10.1111/j.1095-8649.2000.tb00785.x.
- Morgan, M.J., Koen-Alonso, M., Rideout, R.M., Buren, A.D., and Parsons, D.M. 2018. Growth and condition in relation to the lack of recovery of northern cod. ICES J. Mar. Sci. **75**(2): 631–641. doi:10.1093/icesjms/fsx166.
- Morita, S.H., Morita, K., Hamatsu, T., Chimura, M., Yamashita, Y., Sasaki, K., and Sato, T. 2015. Differential effects of the environment on the growth of arabesque greenling (*Pleurogrammus azonus*): does rising temperature benefit young but not old fish? Environ. Biol. Fishes. **98**(3): 801–809. doi:10.1007/s10641-014-0316-x.
- Morley, J.W., Buckel, J.A., and Lankford, T.E. 2012. Comparing Multiple Predictors of Energy Content in Juvenile Bluefish. Trans. Am. Fish. Soc. **141**(4): 1109–1116. doi:10.1080/00028487.2012.675909.
- Nash, R.D.M., Valencia, A.H., and Geffen, A.J. 2006. The origin of Fulton's condition factor — setting the record straight. Fisheries, **31**(5): 236–238.
- Northeast Fisheries Science Center. 2017. Operational assessment of 19 Northeast Groundfish Stocks, Updated through 2016. US Dept. Commer., Northeast Fish Sci Cent Ref Doc. 17-17. doi:10.7289/V5/RD-NEFSC-17-17.
- O'Brien, L., Burnett, J., and Mayo, R.K. 1993. Maturation of nineteen species of finfish off the Northeast coast of the United States, 1985–1990. NOAA Tech. Rep. NMFS 113.
- Peig, J., and Green, A.J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos, **118**(12): 1883–1891. doi:10.1111/j.1600-0706.2009.17643.x.
- Peig, J., and Green, A.J. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Funct. Ecol. **24**(6): 1323–1332. doi:10.1111/j.1365-2435.2010.01751.x.
- Plante, S., Audet, C., Lambert, Y., and De La Noue, J. 2005. Alternative methods for measuring energy content in winter flounder. N. Am. J. Fish. Manage. **25**(1): 1–6. doi:10.1577/M03-104.1.
- Pothoven, S.A., Ludsin, S.A., Hook, T.O., Fanslow, D.L., Mason, D.M., Collingsworth, P.D., and Van Tassell, J.J. 2008. Reliability of bioelectrical impedance analysis for estimating whole-fish energy density and percent lipids. Trans. Am. Fish. Soc. **137**(5): 1519–1529. doi:10.1577/T07-185.1.
- Press, Y.K., McBride, R.S., and Wuenschel, M.J. 2014. Time course of oocyte development in winter flounder *Pseudopleuronectes americanus* and spawning seasonality for the Gulf of Maine, Georges Bank and southern New England stocks. J. Fish. Biol. **85**(2): 421–445. doi:10.1111/jfb.12431. PMID:24942788.
- Rasmussen, J.B., Krimmer, A.N., Paul, A.J., and Hontela, A. 2012. Empirical relationships between body tissue composition and bioelectrical impedance of brook trout *Salvelinus fontinalis* from a Rocky Mountain Stream. J. Fish Biol. **80**(6): 2317–2327. doi:10.1111/j.1095-8649.2012.03295.x. PMID:22551184.
- Renkawitz, M.D., Sheehan, T.F., Dixon, H.J., and Nygaard, R. 2015. Changing trophic structure and energy dynamics in the Northwest Atlantic: implications for Atlantic salmon feeding at West Greenland. Mar. Ecol. Prog. Ser. **538**: 197–211. doi:10.3354/meps11470.
- Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.
- Robards, M.D., Anthony, J.A., Rose, G.A., and Piatt, J.F. 1999. Changes in proximate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season. J. Exp. Mar. Biol. Ecol. **242**: 245–258. doi:10.1016/S0022-0981(99)00102-1.
- Robins, C.R., and Ray, G.C. 1986. A field guide to Atlantic Coast fishes of North America. Houghton Mifflin, Boston.
- Sardenne, F., Chassot, E., Fouche, E., Menard, F., Lucas, V., and Bodin, N. 2016. Are condition factors powerful proxies of energy content in wild tropical tunas? Ecol. Indic. **71**: 467–476. doi:10.1016/j.ecolind.2016.06.031.
- Schloesser, R.W., and Fabrizio, M.C. 2015. Relationships among proximate components and energy density of juvenile Atlantic estuarine fishes. Trans. Am. Fish. Soc. **144**(5): 942–955. doi:10.1080/00028487.2015.1052557.
- Schloesser, R.W., and Fabrizio, M.C. 2017. Condition indices as surrogates of energy density and lipid content in juveniles of three fish species. Trans. Am. Fish. Soc. **146**(5): 1058–1069. doi:10.1080/00028487.2017.1324523.
- Schreckenbach, K., Knosche, R., and Ebert, K. 2001. Nutrient and energy content of freshwater fishes. J. Appl. Ichthyol. **17**(3): 142–144. doi:10.1046/j.1439-0426.2001.00295.x.
- Scott, I., Selman, C., Mitchell, P.I., and Evans, P. 2001. The use of total body electrical conductivity (TOBEC) to determine body composition in vertebrates. In Body composition analysis of animals: a handbook of non-destructive methods. Edited by J.R. Speakman. Cambridge University Press, Cambridge, UK. pp. 127–160.
- Shulman, G.E., and Love, R.M. 1999. Advances in marine biology — The biochemical ecology of marine fishes — Introduction. Adv. Mar. Biol. **36**: 1–351. doi:10.1016/S0065-2881(08)60150-9.
- Smith, B.E., and Link, J.S. 2010. The Trophic Dynamics of 50 Finfish and 2 Squid Species on the Northeast US Continental Shelf [online]. NOAA Tech. Memo. NMFS NE 216. Available from: National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026, USA, or online from <http://www.nefsc.noaa.gov/nefsc/publications/>.
- Smith, C., and Wootton, R.J. 2016. The remarkable reproductive diversity of teleost fishes. Fish Fish. **17**(4): 1208–1215. doi:10.1111/faf.12116.
- Stevenson, R.D., and Woods, W.A. 2006. Condition indices for conservation: new

- uses for evolving tools. *Integr. Comp. Biol.* **46**(6): 1169–1190. doi:10.1093/icb/icl052. PMID:21672816.
- Stolarski, J.T., Margraf, F.J., Carlson, J.G., and Sutton, T.M. 2014. Lipid and moisture content modeling of amphidromous Dolly Varden using bioelectrical impedance analysis. *N. Am. J. Fish. Manage.* **34**(3): 471–481. doi:10.1080/02755947.2014.880764.
- Vidacek, S., Medic, H., Botka-Petrak, K., Nezak, J., and Petrak, T. 2008. Bioelectrical impedance analysis of frozen sea bass (*Dicentrarchus labrax*). *J. Food Eng.* **88**(2): 263–271. doi:10.1016/j.jfoodeng.2008.02.010.
- Vidacek, S., Medic, H., Marusic, N., Tonkovic, S., and Petrak, T. 2012. Influence of different freezing regimes on bioelectrical properties of Atlantic chub mackerel (*Scomber colias*). *J. Food Process Eng.* **35**(5): 735–741. doi:10.1111/j.1745-4530.2010.00622.x.
- Wei, T., and Simko, V. 2016. corrplot: visualization of a correlation matrix [online]. R package version 0.77. Available from <https://CRAN.R-project.org/package=corrplot>.
- Winton, M.V., Wuenschel, M.J., and McBride, R.S. 2014. Investigating spatial variation and temperature effects on maturity of female winter flounder (*Pseudopleuronectes americanus*) using generalized additive models. *Can. J. Fish. Aquat. Sci.* **71**(9): 1279–1290. doi:10.1139/cjfas-2013-0617.
- Wootton, R.J. 1990. Ecology of teleost fishes. Chapman and Hall, London.
- Wuenschel, M.J., Jugovich, A.R., and Hare, J.A. 2006. Estimating the energy density of fish: the importance of ontogeny. *Trans. Am. Fish. Soc.* **135**(2): 379–385. doi:10.1577/T04-233.1.
- Wuenschel, M.J., Able, K.W., and Byrne, D. 2009. Seasonal patterns of winter flounder *Pseudopleuronectes americanus* abundance and reproductive condition on the New York Bight continental shelf. *J. Fish Biol.* **74**(7): 1508–1524. doi:10.1111/j.1095-8649.2009.02217.x. PMID:20735650.
- Wuenschel, M.J., Able, K.W., Vasslides, J.M., and Byrne, D.M. 2013a. Habitat and diet overlap of 4 piscivorous fishes: variation on the inner continental shelf off New Jersey. *Fish. Bull.* **111**(4): 352–369. doi:10.7755/FB.111.4.5.
- Wuenschel, M.J., McBride, R.S., and Fitzhugh, G.R. 2013b. Relations between total gonad energy and physiological measures of condition in the period leading up to spawning: results of a laboratory experiment on black sea bass (*Centropristis striata*). *Fish. Res.* **138**: 110–119. doi:10.1016/j.fishres.2012.05.012.