Discrimination of the endangered Atlantic Whitefish (*Coregonus huntsmani* Scott, 1987) larvae and juveniles

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ABSTRACT

Hasselman, D.J. and Bradford, R.G. 2012. Discrimination of the endangered Atlantic Whitefish (*Coregonus huntsmani* Scott, 1987) larvae and juveniles. Can. Tech. Rep. Fish. Aquat. Sci. 2993: iii + 24 p.

Atlantic Whitefish (*Coregonus huntsmani*) are an endangered species endemic to Nova Scotia, Canada. Several actions identified in the Atlantic Whitefish recovery strategy require accurate identification of the species early life history stages. While criteria exists for the discrimination of adult specimens, no such criteria is available for larvae or juveniles. Using specimens obtained from the captive mating of wild caught adults, and those made available to this study, we conduct discriminant function analyses on phenetic characters, and incorporate species-specific pigmentation patterns to develop discrimination criteria for early life history stage Atlantic Whitefish, Lake Whitefish (*C. clupeaformis*), and Cisco (*C. artedii*). Quantifiable interspecific differences in the number of pre-anal myomeres, total myomeres, and anal fin ray counts, when combined with dorsal and ventral pigmentation patterns can be used to discriminate larval and juvenile specimens of Atlantic Whitefish, Lake Whitefish, and Cisco. The external characters identified as useful common reference points for the delineation of these three species could be extended to include additional coregonine species.

RÉSUMÉ

Le corégone d'Acadie (*Coregonus huntsmani*) est une espèce en voie de disparition endémique à la Nouvelle-Écosse. Plusieurs actions identifiées dans sa stratégie de rétablissement requièrent l'identification précise des premiers stades de vie de l'espèce. Bien qu'il existe des critères pour la différenciation des spécimens adultes, il n'existe pas de tels critères pour les larves et les poissons juvéniles. En utilisant des spécimens obtenus de l'élevage d'adultes capturés en milieu naturel, et d'autres spécimens qui ont été fournis à notre étude, nous utilisons des fonctions discriminantes sur les caractères phénétiques, et incluons des tendances de pigmentation au niveau de l'espèce afin de développer des critères de différenciation pour les premiers stades de vie du corégone d'Acadie, du grand corégone (*C. clupeaformis*) et du Cisco de lac (*C. artedii*). Les différences inter-spécifiques quantifiables du nombre de myomères pré-anal, du nombre total de myomères et du nombre de rayures des nageoires anales, quand elles sont combinées avec les tendances de pigmentation dorsales et ventrales peuvent être utilisées pour discerner les spécimens larvaires et juvéniles du corégone d'Acadie, du grand corégone et du Cisco de lac. Les caractères externes identifiés comme étant d'utiles références communes pour la distinction de ces trois espèces pourrait être étendus à d'autres espèces corégones.

INTRODUCTION

The early life history stages of most freshwater fish species represent several life-intervals that are ecologically distinct from their adult counterparts (Snyder and Muth 1990). Larvae and juveniles often consume different prey, occupy different habitats, and may behave quite differently than conspecific adults. Therefore, knowledge of the habitat requirements, population dynamics, and behaviour of larvae and juveniles has value in the contexts of resource conservation and habitat management, particularly so when endangered species are considered (Snyder and Muth 1990). Accurate identification of target species, irrespective of life-stage, is implicit in any management strategy. Because visual inspection is the first, and frequently the sole opportunity to indicate probable presence of protected species, visual criteria that allow for their accurate identification at all life-stages remain a valuable tool.

Atlantic Whitefish (*Coregonus huntsmani* Scott, 1987) are endemic to Canada, and have a confirmed historic distribution that is limited to only two small coastal drainages located within the Province of Nova Scotia, Canada (Scott and Scott 1988; Bradford et al. 2004). Atlantic Whitefish are listed and protected as an endangered species under Canada's *Species at Risk Act* (*SARA*). Several actions that are considered to be essential to the recovery of Atlantic Whitefish (Department of Fisheries and Oceans (DFO) 2006) require their accurate identification. Multiple external characters have recently been shown by Hasselman et al. (2009) to aid in the discrimination of adult and sub-adult Atlantic Whitefish from other coregonid species occurring within eastern Canada. However, these same characters may not be reliable for the identification of early life history stages because of species-specific differences in ontogenetic development (Fudge et al. 1986; Hasselman et al. 2007). Morphological differences among early life history stages of Atlantic Whitefish, Lake Whitefish, and Cisco have been documented by Hasselman et al. (2007), but their usefulness in species identification has not been assessed. Using discriminant function analysis, we assess whether morphometric and meristic traits in combination with species specific pigmentation patterns can be used to develop an identification key for Atlantic Whitefish, Lake Whitefish and Cisco larvae and juveniles. The framework established in this study could have application beyond the species examined herein, and could potentially be incorporated into existing identification keys established for early life history stages of other coregonine species.

MATERIALS AND METHODS

SPECIMEN PROCUREMENT/PREPARATION

Atlantic Whitefish eggs, larvae, and juveniles acquired from the captive mating of wild-caught adults at the DFO Mersey Biodiversity Facility, Milton, N.S. were sampled as a time series as described in Hasselman et al. (2007). Lake Whitefish specimens reared from known parental stock at White's Lake Fish Culture Station in Sharbot Lake, Ontario, were supplemented with wild-caught Lake Whitefish and Cisco specimens from Southern Indian Lake, Manitoba which were made available to this study (see Fudge et al. 1986). Although morphological characters for early life stage freshwater fishes may exhibit intraspecific spatial and temporal variation

(Bosley and Conner 1984), the absence of hatcheries for Lake Whitefish and Cisco in the Canadian Maritime Provinces necessitated this approach.

Freshly acquired specimens were fixed in 10% formalin for two weeks prior to examination, while the reference collections of Cisco and Lake Whitefish (see Fudge et al. 1986) were fixed in 10% formalin for an extended period of time (i.e. ~20 years).

LABORATORY MEASUREMENTS/PROCEDURES

Specimens were assigned to one of four discrete developmental stages: i) yolk-sac larvae (hatch until yolk absorption completed; Kendal et al. 1984), ii) pre-flexion larvae (post-yolk absorption until notochord flexion, evidence of principal/secondary caudal fin rays; Hardy et al. 1978), iii) post-flexion larvae (completion of notochord flexion; Ahlstrom 1976), and iv) juvenile (adult complement of fin rays in all rayed fins, and attainment of adult body proportions/pigmentation; Mansueti and Hardy 1967).

Specimens were left-side photographed with a 35mm Nikon N2000 SLR camera mounted to a Nikon SMZ1000 stereo dissecting microscope, or macroscopically with a tripod and using a 50 mm macro lens. Pigmentation patterns were characterized in terms of the organizational scheme and density of melanophores along the dorsal, ventral, and lateral surfaces for each species at each of the four stages. Eleven morphometric characters were measured (nearest 0.01 mm) and eight meristic characters (Table 1) were counted from specimen photographs using Axiovision image analysis software (Imaging Associates Limited, Bicester, Oxfordshire, UK). Following Hasselman et al. (2007), the morphometric and meristic characters examined in this study were as follows: yolk sac length, depth, and width, body depth at yolk sac, lower jaw length (Hasselman 2003), preanal length (Koelz 1929), eye diameter (Bodaly 1979), head length and depth (Casselman et al. 1981), upper jaw length, snout length (Hubbs and Lagler 1958), and number of preanal, postanal, and total myomeres (Hasselman 2003), number of caudal, anal, dorsal, pelvic, and pectoral fin rays (Hubbs and Lagler 1958).

STATISTICAL PROCEDURES

Morphometric variables were $\log_{10}$ transformed to achieve normality (Krzanowski 1988) without affecting the allometries (Jolicouer 1963). Normality of the transformed data was confirmed via the Kolmogorov-Smirnov one-sample test (SYSTAT v. 10.0; SPSS, Inc. 2000). Residual values expressing variation in morphometric measures independent of size effects were calculated for all morphometric characters as recommended by Reist (1985, 1986) and following the method of Moore and Bronte (2001). Briefly, each $\log_{10}$ transformed variable was regressed on the first principal component of all morphometric variables from all specimens (factor analysis routine of SYSTAT) (dos Reis *et al*., 1990) for each larval/juvenile stage. The calculation of residuals is not required for meristic characters. Residuals were inspected as box-plots to identify outliers, with those that could not be attributed to data entry error assumed to reflect natural variability, and included in analyses. Regression analysis of all transformed morphometric characters on specimen total length verified that size effects had been eliminated.

Discriminant function analysis (DFA; discriminant analysis routine of SYSTAT) was conducted separately for each discrete larval/juvenile stage using an interactive forward stepwise procedure ($F_{\geq 4}$-to-enter inclusion criterion). An interactive backward stepwise procedure was implemented when 15 or fewer variables were considered, as recommended by Johnson (1998). Tolerance level was set to 1.0 to minimize co-linearity between variables.

RESULTS

Although several morphometric characters (e.g. pre-anal length, head depth, upper jaw length) met variable inclusion criteria in DFA, a high degree of morphological similarity among early life history stage coregonines rendered discrimination based on morphometric characters equivocal. Therefore, we report only those characters which appreciably aided species identification. These characters were used in concert with pigmentation patterns to construct a polytomous species identification key. However, due to broad interspecific overlap in the distributions of values for these traits we constructed a 'non-linear' key by incorporating additional criteria at various steps in the identification process to aid species discrimination.

YOLK-SAC LARVAE

Reliable species identification of yolk-sac larvae required a combination of myomere counts, as well as dorsal and ventral pigmentation patterns. Preanal myomeres was the most diagnostic character, and correctly re-assigned all Atlantic Whitefish and Cisco, and 69% of Lake Whitefish to species (jack-knifed cross validation; Table 2). Atlantic Whitefish possessed a significantly ($p<0.05$; two-sample separate variance t-test; SYSTAT) greater number of preanal myomeres (40-44) than Cisco (33-36) or Lake Whitefish (32-41) (Table 2; Figure 1a). However, there was some overlap in the distribution of preanal myomeres for Lake Whitefish with both Cisco, and Atlantic Whitefish. Total myomeres also aided species identification, but was not a diagnostic trait (Table 2). Atlantic Whitefish possessed a significantly ($p<0.05$; two-sample separate variance t-test; SYSTAT) greater number of total myomeres (57-64) than Cisco (53-57) or Lake Whitefish (48-60) (Table 2; Figure 1b). Cumulatively, preanal and total myomere counts correctly re-assigned 99% of Atlantic Whitefish, 79% of Lake Whitefish, and all Cisco to species (Table 2).

Dorsal pigmentation for Atlantic Whitefish consisted of melanophores arranged in two distinct bilaterally symmetrical rows, one on either side of the median finfold that extended the length of the body (Figure 2b). Dorsal melanophores for Cisco and Lake Whitefish were sparsely distributed over the length of the body. Ventral pigmentation for Atlantic Whitefish consisted of a dense concentration of melanophores between the pectoral fin buds and on the yolk-sac, and two rows of melanophores, one on either side of the pre-anal finfold, that extended posteriorly to the vent where they intersected and continued to the caudal region (Figure 2c). Cisco exhibited comparatively little ventral pigmentation, with melanophores sparsely distributed over the yolk-sac and between the pectoral fin buds. Lake Whitefish exhibited melanophores either restricted to the yolk-sac, or sparsely distributed over the yolk-sac, around the vent, and between the vent

and caudal fin. A key to coregonine yolk-sac larvae is presented below with an accompanying schematic in Figure 3 depicting relevant species specific pigmentation patterns.

Key to yolk-sac larvae

1 Pre-anal myomere count ≥ 42……...……...Atlantic Whitefish

Pre-anal myomere count 32 or 37-39.…….……..Lake Whitefish

Pre-anal myomere count 33-36 or 40-41…….……2

2 Total myomere count ≥ 61…….……...……...Atlantic Whitefish

Total myomere count ≤ 52……..……..Lake Whitefish

Total myomere count 53-60…….……3

3 Dorsal pigmentation arranged as two distinct bilaterally symmetrical rows, one on either side of the median finfold, and extending length of body……..Atlantic Whitefish

Dorsal pigmentation sparsely distributed over body……...……4

4 Ventral pigmentation sparsely distributed over yolk-sac, and between pectoral fin buds…….…….…….....Cisco

Ventral pigmentation restricted to yolk-sac, or on yolk-sac, around vent, and between vent and caudal fin……..…….Lake Whitefish

PRE-FLEXION LARVAE

A combination of myomeres counts, as well as dorsal and ventral pigmentation patterns provided reliable species identification criteria for pre-flexion larvae. Preanal myomeres correctly re-assigned 97% of Atlantic Whitefish, 79% of Lake Whitefish, and 87% of Cisco pre-flexion larvae to species (jack-knifed cross validation; Table 2). Atlantic Whitefish possessed a significantly ($p<0.05$; two-sample separate variance t-test; SYSTAT) greater number of pre-anal myomeres (39-43) than either Cisco (35-37), or Lake Whitefish (35-41) (Figure 1c). Total myomeres correctly re-assigned all Atlantic Whitefish, and 63% of Cisco and Lake Whitefish specimens (jack-knifed cross validation) to species (Table 2). Atlantic Whitefish possessed a significantly ($p<0.05$; two-sample separate variance t-test; SYSTAT) greater number of total myomeres (61-65) than Cisco (52-58) or Lake Whitefish (53-60) (Table 2; Figure 1d). Cumulatively, preanal and total myomere counts correctly re-assigned 100% of Atlantic Whitefish, 84% of Lake Whitefish, and 90% of Cisco to species (Table 2).

Atlantic Whitefish and Lake Whitefish pre-flexion larvae both possessed bilaterally symmetrical rows of melanophores along the dorsal surface that extended the length of the body (Figure 4b). However, Cisco possessed sporadically distributed melanophores on either side of the median finfold that were largely isolated to posterior of the dorsal fin. Atlantic Whitefish ventral pigmentation was observed between the pectoral fins and formed a line which faded posteriorly as melanophores became more sparsely distributed (Figure 4c). A bilateral row of ventral pigmentation was evident posterior of the pelvic fin buds, but terminated with their intersection posterior of the insertion of the anal fin. Lake Whitefish either lacked ventral pigmentation entirely, or possessed an aggregation of melanophores isolated between the pectoral fins. Cisco possessed comparatively little ventral pigmentation, as sporadically distributed melanophores were restricted to the vent region. A key to coregonine pre-flexion larvae is presented below with an accompanying schematic in Figure 5 depicting relevant species specific pigmentation patterns.

Key to pre-flexion larvae

1 Total myomere count $\geq$ 61……..……...……...Atlantic Whitefish

Total myomere count 52……...……Cisco

Total myomere count 59-60……..…...Lake Whitefish

Total myomere count 53-58…….2

2 Pre-anal myomere count $\geq$ 42……...……...Atlantic Whitefish

Pre-anal myomere count 38……....……..Lake Whitefish

Pre-anal myomere count 35-37 or 39-41…….……3

3 Ventral pigmentation between pectoral fins forms a line which fades posteriorly as melanophores become sparsely distributed; bilateral row of ventral pigmentation evident posterior of pelvic fin buds and terminates posterior of the insertion of the anal fin…….…....Atlantic Whitefish

Ventral pigmentation restricted to area around vent……...……..Cisco

Ventral pigmentation either isolated between pectoral fins not extending posteriorly, or generally absent…….……...……4

4 Dorsal pigmentation arranged as two distinct bilaterally symmetrical rows, one on either side of the median finfold, and extending length of body…….....Lake Whitefish

Dorsal pigmentation sporadically distributed over body……...….... ...Cisco

POST-FLEXION LARVAE

Increased body pigmentation and thickening of the body wall prohibited accurate assessment of myomere counts for post-flexion larvae. Reliable species identification required the combined use of anal fin ray counts, as well as ventral and lateral pigmentation patterns. Anal fin rays correctly re-assigned only 31% of Atlantic Whitefish, 63% of Cisco, and no Lake Whitefish to species (jack-knifed cross validation; Table 2). Although anal fin rays were not diagnostic, Lake Whitefish possessed significantly ($p<0.05$; two-sample separate variance t-test; SYSTAT) fewer anal fin rays (9-14) than either Atlantic Whitefish (12-14), or Cisco specimens (12-16) (Table 2). Differences in anal fin ray counts between Atlantic Whitefish and Cisco were non-significant ($p>0.05$).

Atlantic Whitefish ventral pigmentation was observed between the pectoral fins as a line of melanophores that extended posteriorly about 1/3 the distance to the insertion of the pelvic fins, after which melanophores were sporadically distributed posteriorly. A dense aggregation of melanophores extended from the pelvic fins, around the vent, and terminated near the insertion of the caudal fin (Figure 6c). Cisco lacked ventral pigmentation entirely. Lake Whitefish possessed either sporadically distributed melanophores between the pectoral and pelvic fins, or a 'V-shaped' band of pigmentation that originated between the pectoral fins with the 'V' oriented posteriorly. Melanophores were scattered over the remainder of the ventral surface in no definitive pattern. Atlantic Whitefish possessed a completely pigmented lateral line (Figure 6a). Cisco possessed a partially pigmented lateral line, from the dorsal to caudal fin. Lake Whitefish either lacked lateral line pigment entirely, or possessed a partially pigmented lateral line, from the dorsal to caudal fin. A key to coregonine post-flexion larvae is presented below with an accompanying schematic in Figure 7 depicting relevant species specific pigmentation patterns.

Key to post-flexion larvae

1 Number of anal fin rays ≤ 11……..…….…….……Lake Whitefish

Number of anal fin rays ≥15……...……..…..……..….Cisco

Number of anal fin rays 12-14…….2

2 Fully pigmented lateral line……..Atlantic Whitefish

Lateral line partially pigmented (caudal fin to dorsal fin) or non-pigmented……..…3

3 Ventral pigmentation between pectoral fins in a narrow line extending 1/3 the distance to pelvic fins, gradually fading posteriorly…….Atlantic Whitefish

Ventral pigmentation localized between pectoral fins 'V-shaped'…….....Lake Whitefish

Ventral pigmentation absent…….……...…..…Cisco

JUVENILES

Reliable identification of juvenile specimens required the combined use of anal fin rays and ventral pigmentation patterns. Anal fin rays correctly re-classified 69, 81, and 44% of Atlantic Whitefish, Lake Whitefish, and Cisco, respectively (Table 2; jack-knifed cross validation). Although not a diagnostic character, Lake Whitefish possessed significantly ($p<0.05$) fewer anal fin rays (10-14) than either Atlantic Whitefish (12-15) or Cisco (12-15) (Table 2).

Atlantic Whitefish ventral pigmentation consisted of a band of melanophores isolated between the pectoral fins that gradually dissipated as it extended posteriorly (Figure 8c). Lake Whitefish also possessed a band of melanophores isolated between the pectoral fins that terminated abruptly as it extended posteriorly, but was greater in width than that observed for Atlantic Whitefish. Cisco lacked ventral pigmentation between the pectoral fins, but melanophores were observed around the vent, and on either side of the anal fin. Melanophores were not observed near the vent or anal fin region in Lake Whitefish. A key to coregonine juveniles is presented below with an accompanying schematic in Figure 9 depicting relevant species specific pigmentation patterns.

Key to juveniles

1 Number of anal fin rays ≤ 11……..…….……..Lake Whitefish

Number of anal fin rays ≥12……..…..……..……...2

2 Ventral pigmentation in a narrow band originating between pectoral fins, and gradually dissipating posteriorly. Pigmentation around the vent and on either side of the anal fin……...……..…...Atlantic Whitefish

Ventral pigmentation originating between pectoral fins ends abruptly as it extends posteriorly. No pigmentation around the anal fin region…....Lake Whitefish

No ventral pigmentation originating between the pectoral fins, but localized around the vent and on either side of the anal fin…….....……Cisco

DISCUSSION

No single morphometric or meristic character examined in this study was diagnostic in the discrimination of early life history stage coregonines. Reliable species identification required the combined use of multiple traits. Species specific differences in myomere counts (preanal and total), anal fin ray counts, and pigmentation patterns provide the basis for discrimination among early life history stage Atlantic Whitefish, Lake Whitefish, and Cisco.

Among yolk-sac and pre-flexion larvae, Atlantic Whitefish possess a greater number of pre-anal and total myomeres than either Cisco or Lake Whitefish (Table 2; Figure 1). Differences in myomere counts are consistent with the greater number of vertebrae reported for Atlantic Whitefish (64-67) than for Lake Whitefish from the Canadian Maritime Provinces (58-64); a diagnostic character in the discrimination of adult specimens (Edge et al. 1991). Considering that vertebrae develop from the sclerotomes of the [myomeres] (Richardson et al. 1998), and that vertebrae number is fixed at or shortly after emergence (Lindsey 1975), interspecific differences in myomere counts provide valuable morphological criteria for coregonid species identification in Atlantic Canada.

Among post-flexion larvae and juvenile specimens, Lake Whitefish possess significantly fewer anal fin rays than either Atlantic Whitefish, or Cisco (Table 2). Although anal fin ray counts do not discriminate Atlantic Whitefish and Cisco, consistent interspecific differences in ventral pigmentation pattern are valuable for species identification.

Interspecific differences in pigmentation patterns (Hart 1930; Pritchard 1930; Lindstrom 1962; Faber 1970; Hinrichs 1979; Cucin and Faber 1985; Fudge et al. 1986), colouration (Hart 1930), and melanophore size (Hinrichs 1979; Auer 1982) have proven reliable criteria for identification of coregonid species. We did not examine colouration because of concerns over the confounding effects that prolonged preservation (~20 years) may have had on specimen colour (i.e. Cisco specimens), and subsequent application to *in situ* species identification. Similarly, we did not examine differences in melanophore size due to concerns over their contraction/expansion in response to factors such as varying intensities of incident light (Johnston 1984). Therefore, our assessment of interspecific pigmentation patterns considers only the *distribution* of melanophores about the body. Consistent species specific patterns of melanophore distribution identified herein provide valuable criteria for the discrimination of larval and juvenile Atlantic Whitefish, Lake Whitefish, and Cisco.

Our comparison of preserved cultured and fresh specimens of both Cisco and Lake Whitefish from a non-local source with Atlantic Whitefish is one of necessity. Hatcheries in the Canadian Maritime Provinces do not culture either species, and cannot provide early life history stages from local source populations. Given the potential for spatial and temporal intraspecific phenotypic variation (Bosley and Conner 1984), the extent to which the use of non-local source populations restricts the application of the characters identified herein for *in situ* species identification is uncertain. However, the interspecific differences identified in this study may retain their value in species identification. For example, while vertebral counts for Lake Whitefish from the Maritime Provinces (58-64; Edge et al. 1991) slightly exceed total myomeres for Lake Whitefish larvae ($\geq$60) examined herein, Atlantic Whitefish possess an even greater number of vertebrae (64-67; Edge et al. 1991). Given the previously noted relationship between myomeres and vertebrae (Richardson et al. 1998), myomere counts probably provide a useful character for species identification in Atlantic Canada. Anal fin ray counts remain valuable in species discrimination as non-significant ($p<0.05$) differences were observed for the number of anal fin rays observed among Lake Whitefish examined herein, and adult Lake Whitefish from the Maritime Provinces (n=640; data not shown). Fudge et al. (1986) found that accurate discrimination of Lake Whitefish and Cisco from both preserved stock and wild specimens was possible using pigmentation patterns. We anticipate that the species specific differences in

pigmentation patterns identified in this study will retain their value for *in situ* identification of larval coregonids from the Maritime Provinces.

This study contributes to Atlantic Whitefish biology, and to the body of knowledge concerning the discrimination of freshwater fish early life history stages - an area of research which is incomplete for the majority of North American species (Snyder and Muth 1990). Discrimination criteria is required to support several initiatives identified in the Atlantic Whitefish recovery strategy (DFO 2006), and this study establishes morphological criteria that can be used to reliably identify early life history stage coregonids in Atlantic Canada, and resolves the absence of Atlantic Whitefish from early life history fish keys. Revision of this information using early life history stage Lake Whitefish from the Maritime Provinces would help to refine the morphological criteria identified herein.

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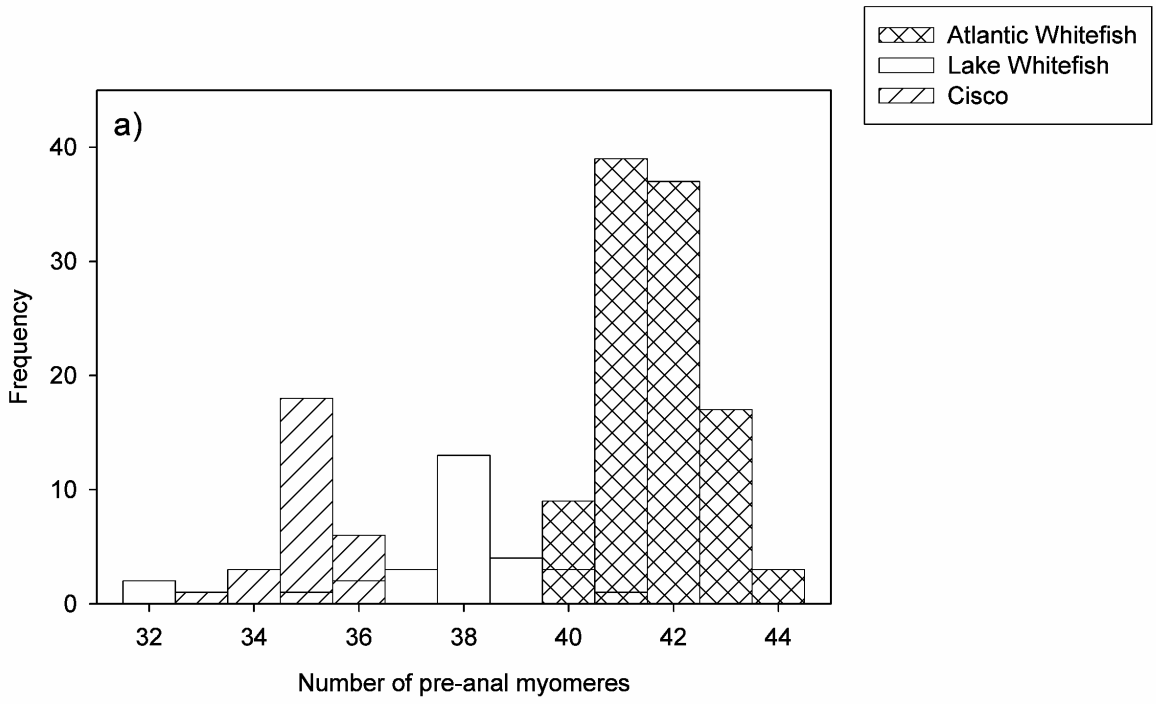
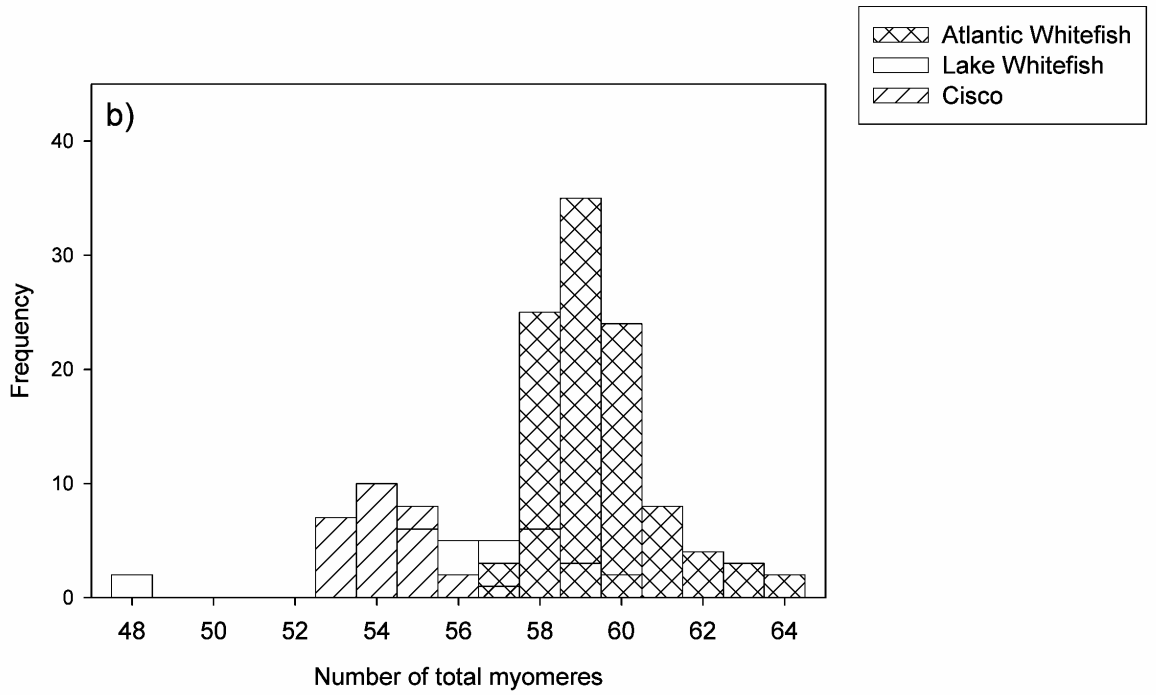

Atlantic Whitefish
Lake Whitefish
Cisco
a)
Frequency
0
10
20
30
40
32
34
36
38
40
42
44
Number of pre-anal myomeres


Atlantic Whitefish
Lake Whitefish
Cisco
b)
Frequency
0
10
20
30
40
48
50
52
54
56
58
60
62
64
Number of total myomeres

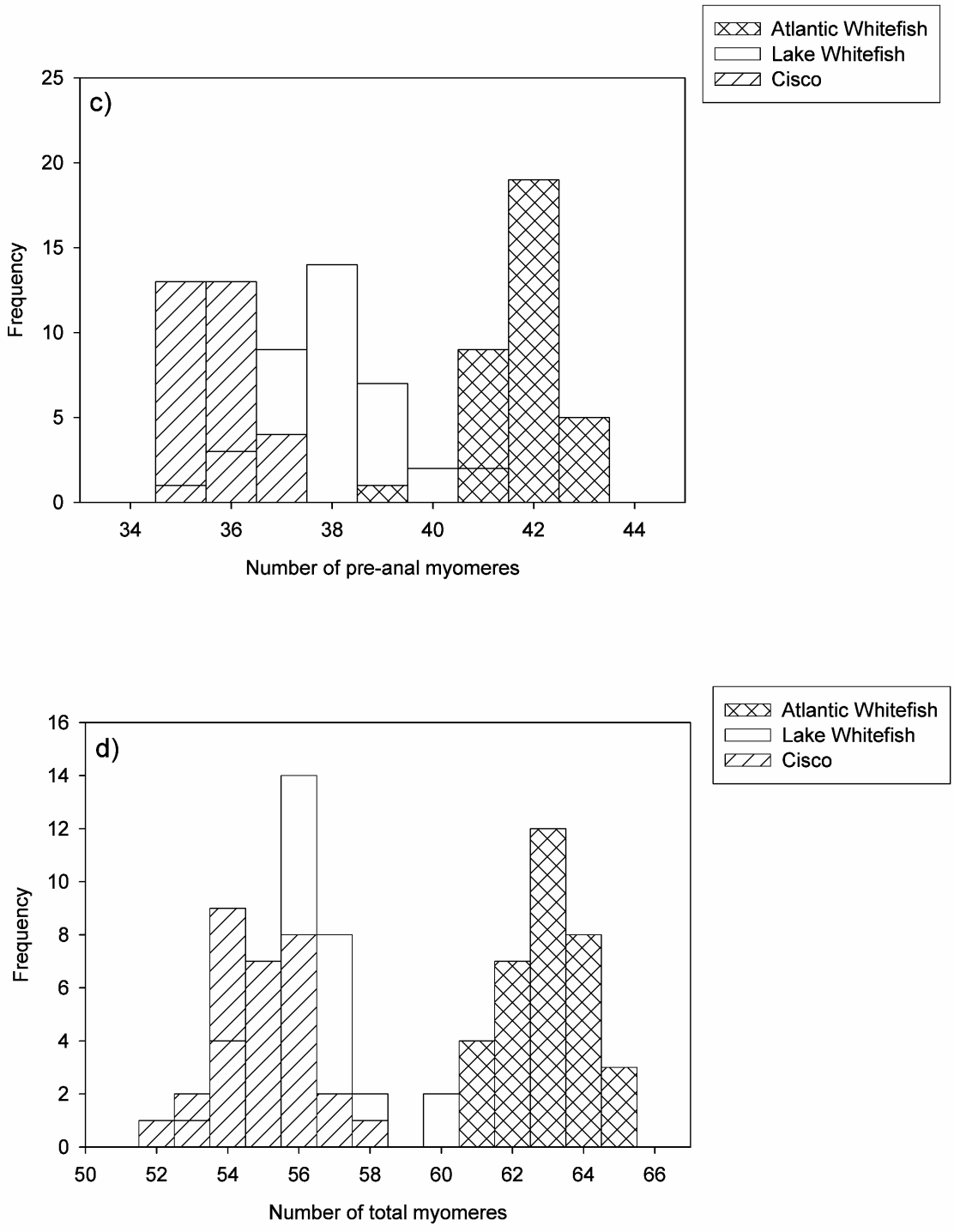


Figure 1: Distribution of preanal myomere and total myomere counts among Atlantic Whitefish, Lake Whitefish and Cisco yolk-sac larvae (a,b) and pre-flexion larvae (c,d).

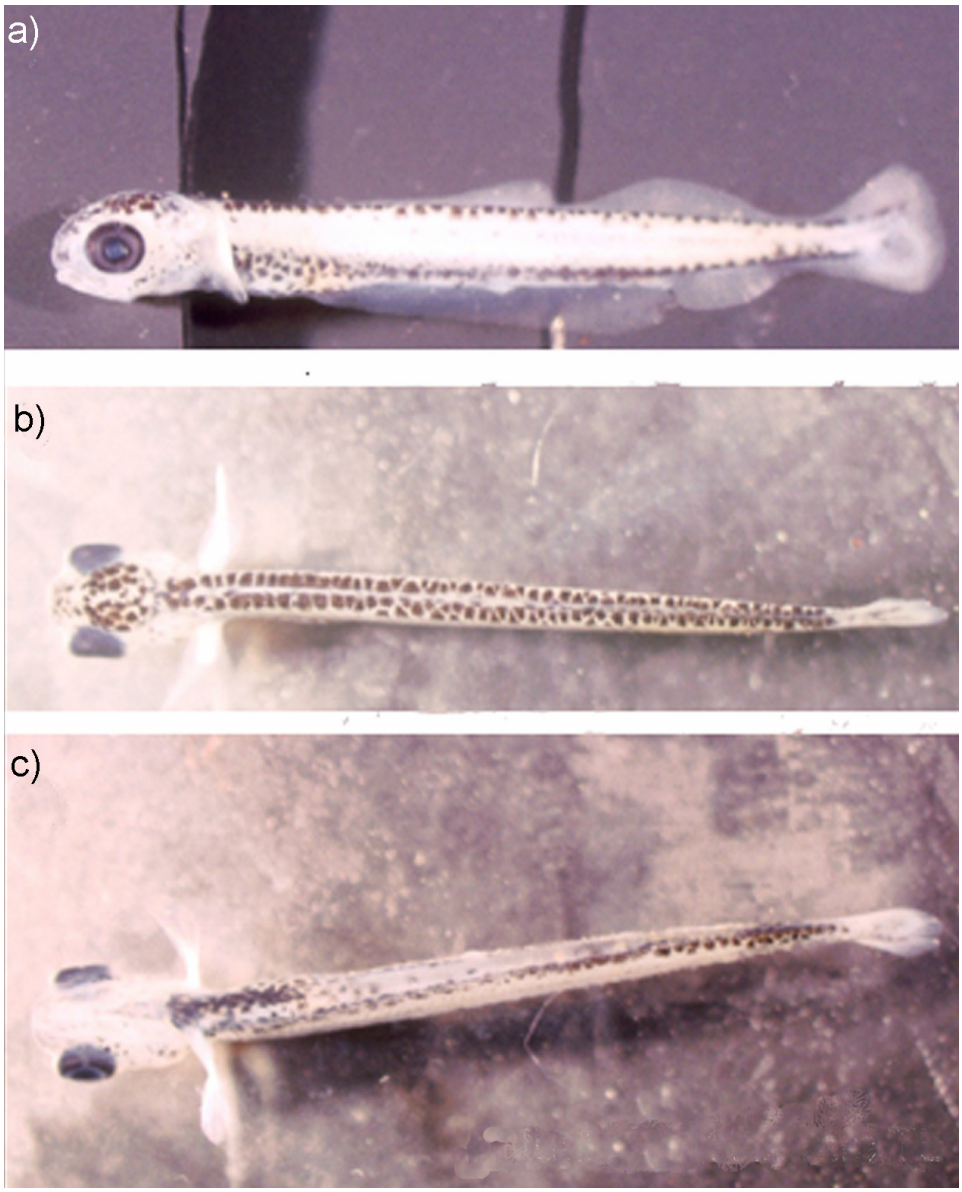


Figure 2: Photographs of Atlantic Whitefish yolk-sac larvae a) lateral, b) dorsal, and c) ventral pigmentation patterns; 15.2mm TL.

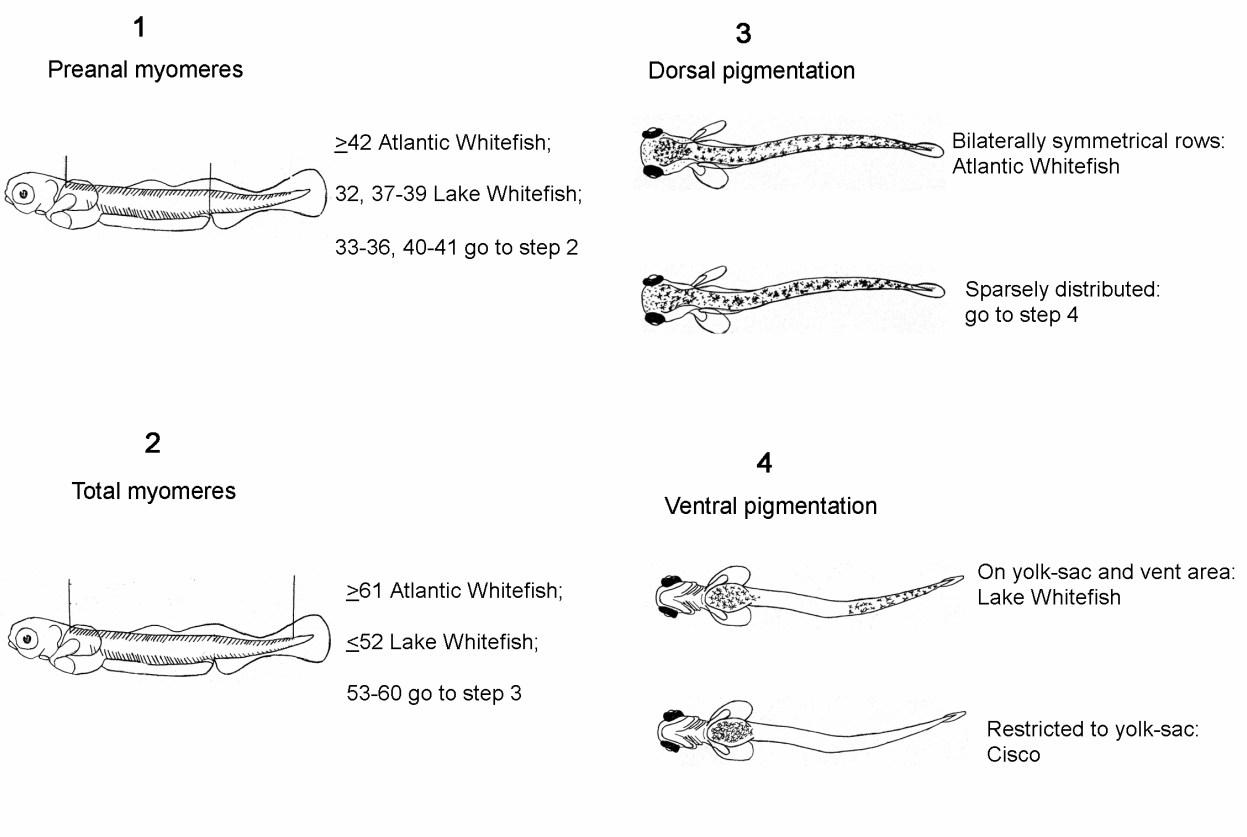


Figure 3: Schematic of relevant meristic characters and pigmentation patterns required for accurate species identification of coregonine yolk-sac larvae in Atlantic Canada.

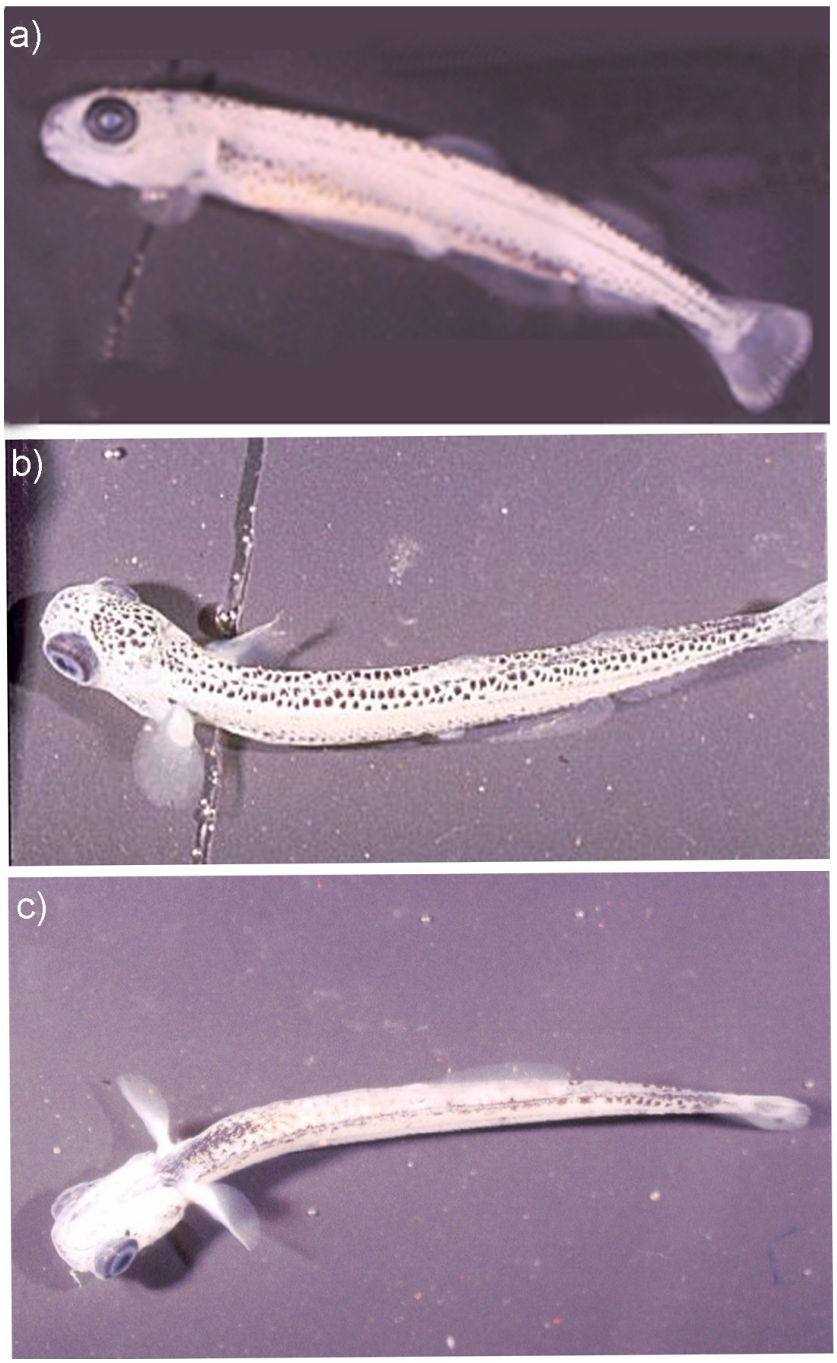


Figure 4: Photographs of Atlantic Whitefish pre-flexion larvae a) lateral, b) dorsal, and c) ventral pigmentation patterns; 22.0mmTL.

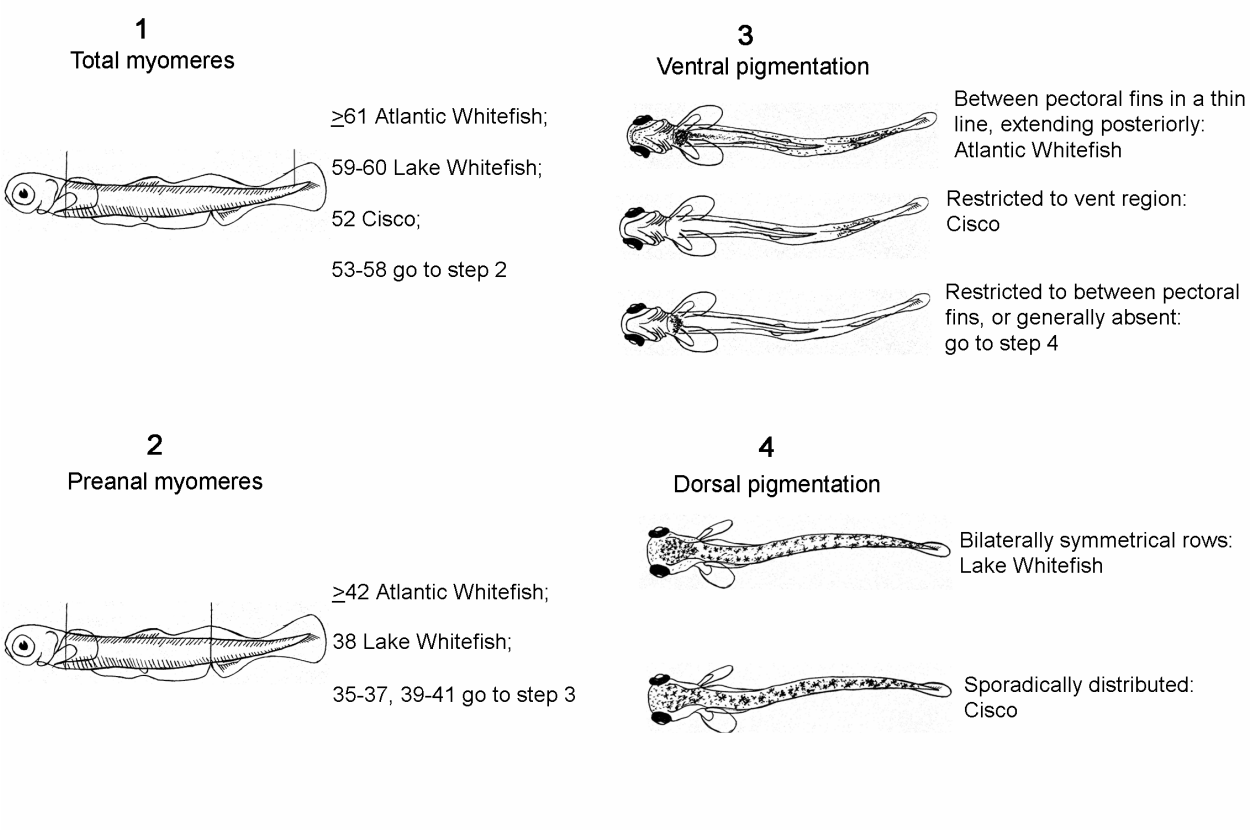


Figure 5: Schematic of relevant meristic characters and pigmentation patterns required for accurate species identification of coregonine pre-flexion larvae in Atlantic Canada.

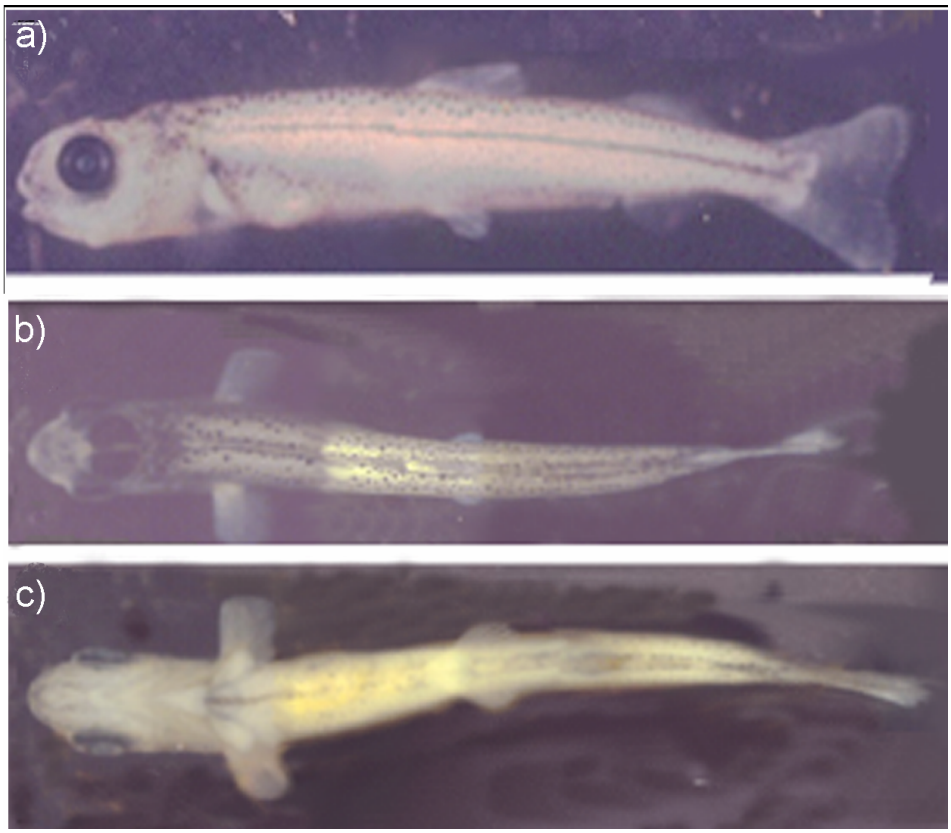


Figure 6: Photographs of Atlantic Whitefish post-flexion larvae a) lateral, b) dorsal, and c) ventral pigmentation patterns; 32.7 mm TL.

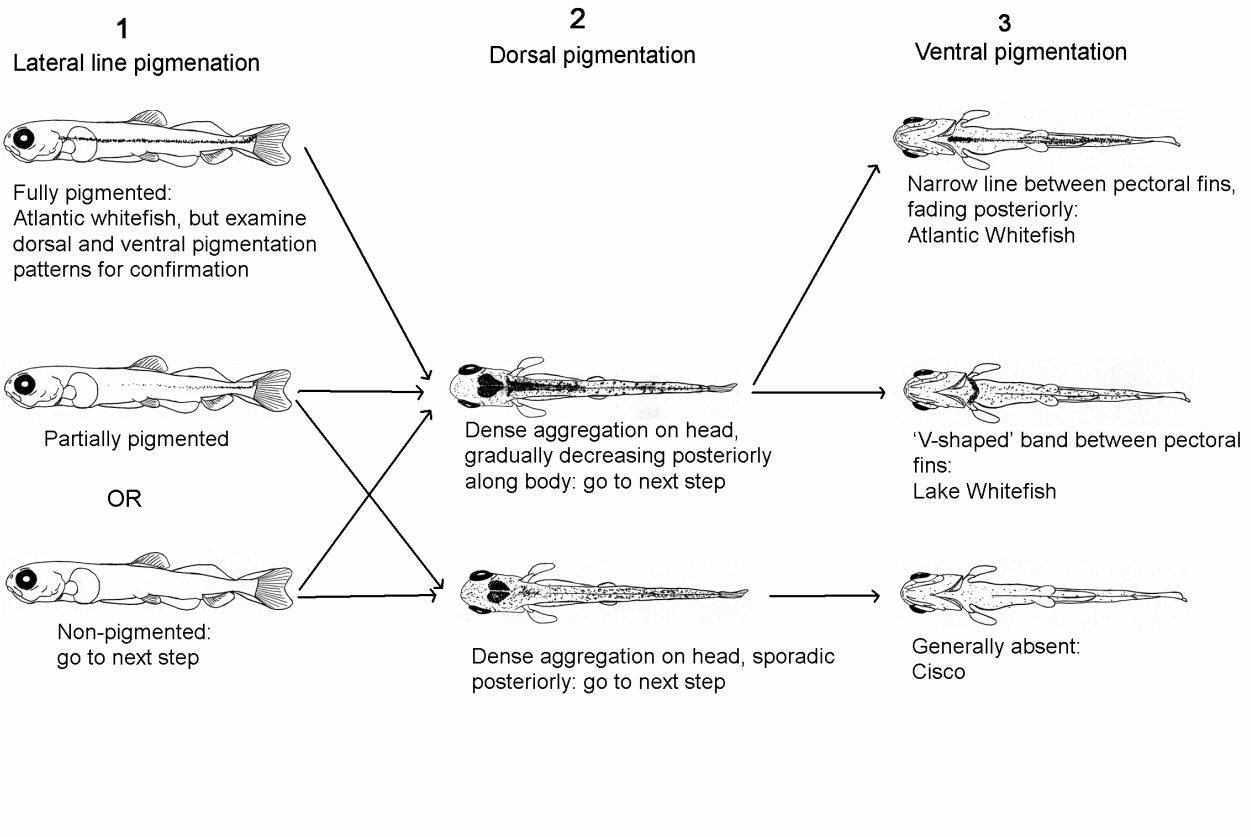


Figure 7: Schematic of relevant pigmentation patterns required for accurate species identification of coregonine post-flexion larvae in Atlantic Canada.

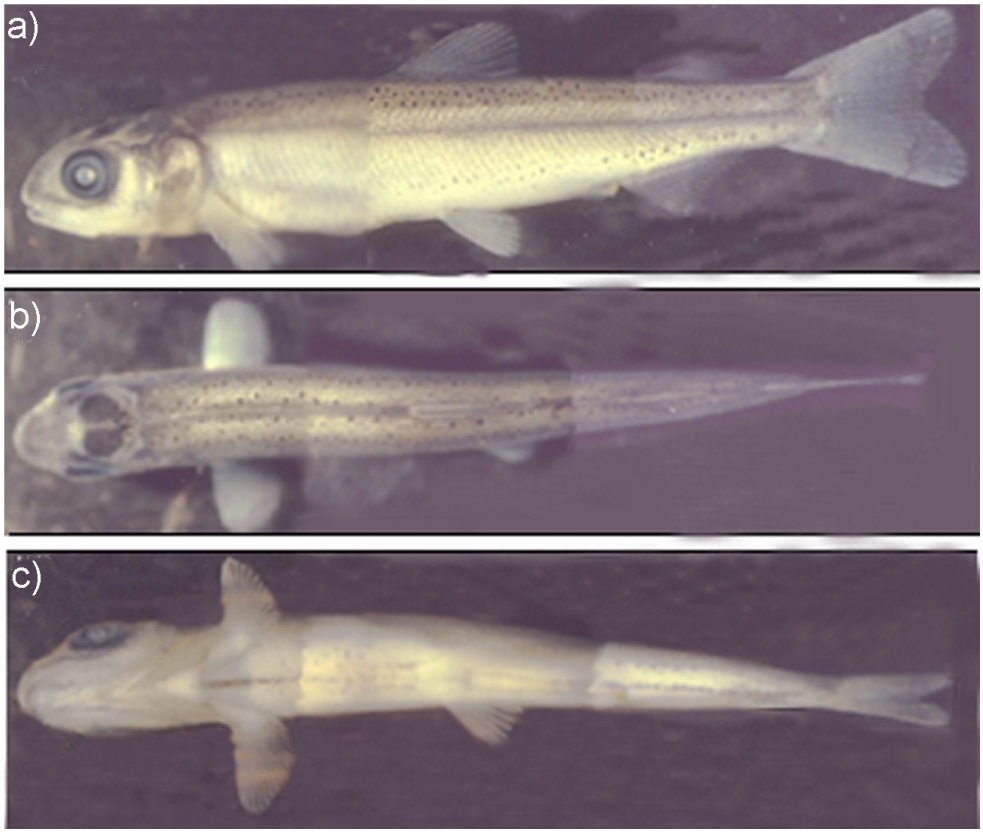


Figure 8: Photographs of Atlantic Whitefish juvenile a) lateral, b) dorsal, and c) ventral pigmentation patterns; 39.0 mm TL.

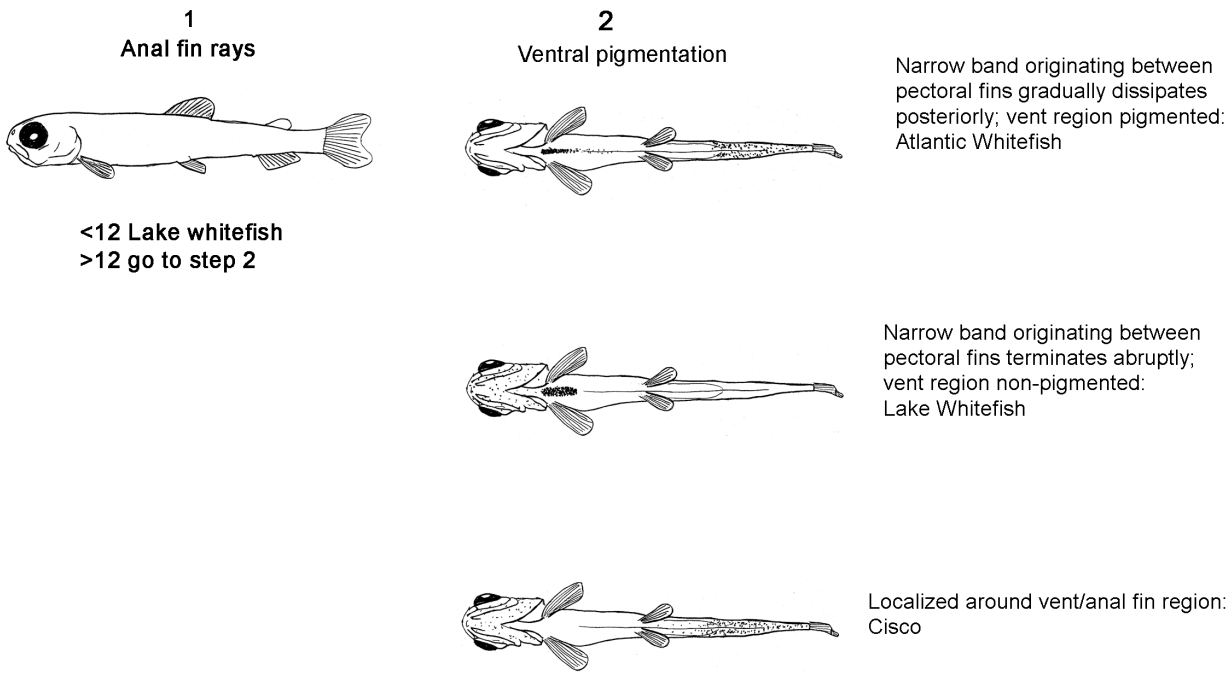


Figure 9: Schematic of ventral pigmentation patterns required for accurate species identification of juvenile coregonines in Atlantic Canada.

Table 1: Eleven morphometric and eight meristic characters examined for early life history stage Atlantic Whitefish, Lake Whitefish, and Cisco.

Variable	**Early life history stage**			
	Yolk-sac larvae	**Pre-flexion larvae**	**Post-flexion larvae**	**Juvenile**
Yolk-sac length	✓	NO	NO	NO
Yolk-sac width	✓	NO	NO	NO
Yolk-sac depth	✓	NO	NO	NO
Head length	✓	✓	✓	✓
Head depth	✓	✓	✓	✓
Eye diameter	✓	✓	✓	✓
Upper jaw length	✓	✓	✓	✓
Lower jaw length	✓	✓	✓	✓
Snout length	✓	✓	✓	✓
Body depth at anus	✓	✓	✓	✓
Pre-anal length	✓	✓	✓	✓
Number of pre-anal myomeres	✓	✓	NO	NO
Number of post-anal myomeres	✓	✓	NO	NO
Total myomeres	✓	✓	NO	NO
Number of caudal fin rays	ND	✓	✓	✓
Number of dorsal fin rays	ND	✓	✓	✓
Number of pectoral fin rays	ND	ND	✓	✓
Number of pelvic fin rays	ND	ND	✓	✓
Number of anal fin rays	ND	✓	✓	✓

Note: ND, not yet developed; NO, not observable

Table 2: Mean values for external characters that discriminate Atlantic Whitefish, Lake Whitefish, and Cisco for each of four ontogenetic stages. Percent correct re-assignment (jack-knifed cross validation) provided by characters when considered individually are shown. Aggregate classification refers to the cumulative degree of correct re-assignment when characters are considered simultaneously.

Character		Ontogenetic stage											
		Yolk sac larvae			Preflexion larvae			Postflexion larvae			Juvenile		
		Atlantic Whitefish	Lake Whitefish	Cisco	Atlantic Whitefish	Lake Whitefish	Cisco	Atlantic Whitefish	Lake Whitefish	Cisco	Atlantic Whitefish	Lake Whitefish	Cisco
Pre-anal	Range	40-44	32-41	33-36	39-43	35-41	35-37	NO	NO	NO	NO	NO	NO
myomeres	Mean	41.68	37.69	35.04	41.79	37.97	35.7	-	-	-	-	-	-
	(±sd)	(0.95)	(2.02)	(0.69)	(0.81)	(1.30)	(0.70)						
	N	105	29	28	34	38	30	-	-	-	-	-	-
	%	100	69	100	97	79	87	-	-	-	-	-	-
Total myomeres	Range	57-64	48-60	53-57	61-65	53-60	52-58	NO	NO	NO	NO	NO	NO
	Mean	59.41	56.41	54.29	62.97	56.05	54.97	-	-	-	-	-	-
	(±sd)	(1.42)	(2.78)	(1.05)	(1.14)	(1.47)	(1.33)						
	N	104	29	28	34	38	30	-	-	-	-	-	-
	%	97	34	89	100	63	63	-	-	-	-	-	-
Anal fin rays	Range	ND	ND	ND	ND	ND	ND	12-14	9-14	12-16	12-15	10-14	12-15
	Mean	-	-	-	-	-	-	13.08	12.17	13.13	13.68	11.92	13.55
	(±sd)							(0.84)	(1.12)	(1.20)	(1.00)	(0.80)	(1.01)
	N	-	-	-	-	-	-	36	30	16	65	37	9
	%	-	-	-	-	-	-	31	0	63	69	81	44
Aggregate	N	104	29	28	34	38	30	NA	NA	NA	NA	NA	NA
c1assification	%	99	79	100	100	84	90	-	-	-	-	-	-

Note: NA-not applicable; ND-not yet developed; NO-not observable.