Use of parasites in stock identification of the deepwater redfish (*Sebastes mentella*) in the Northwest Atlantic

David J. Marcogliese

St. Lawrence Centre
Environment Canada
105 McGill, 7th Floor
Montreal, Québec H2Y 2E7, Canada
E-mail address: david.marcogliese@ec.gc.ca

Elaine Albert

Department of Fisheries and Oceans
Maurice Lamontagne Institute
P.O. Box 1000
Mont-Joli, Québec G5H 3Z4, Canada

Pierre Gagnon

St. Lawrence Centre
Environment Canada
105 McGill, 7th Floor
Montreal, Québec H2Y 2E7, Canada

Jean-Marie Sévigny

Department of Fisheries and Oceans
Maurice Lamontagne Institute
P.O. Box 1000
Mont-Joli, Québec G5H 3Z4, Canada

An important aspect of fisheries management is the correct delineation of boundaries between fish stocks. With the recent collapse of the groundfish fishery in eastern Canada, redfish (*Sebastes* spp.) has become an increasingly important resource. Currently, the two most economically important redfish species (*S. fasciatus* and *S. mentella*) are partitioned into eight management areas in the Northwest Atlantic (Fig. 1). In this study, we examined the parasite fauna of the deepwater redfish *S. mentella*, collected from different areas in the Northwest Atlantic, to determine if the distribution and abundance of the parasite fauna can aid in stock discrimination of this species and focused on differentiating fish from the Gulf of St. Lawrence (unit 1) from those from the Cabot Strait and Laurentian Channel (unit 2). The deepwater redfish is the most common redfish species in the Gulf of St. Lawrence and is considered to have a more northerly range and deeper distribution than its congeners (Atkinson, 1987; Scott and Scott, 1988).

In eastern Canada, parasites have been successfully employed to discriminate among stocks of another deepwater fish, the Greenland halibut (*Reinhardtius hippoglossoides*) (Arthur and Albert, 1993), as well as Atlantic cod (*Gadus morhua*) (McClelland and Marcogliese, 1994). Moles et al. (1998) suggested that parasites could be used to discriminate among stocks of rockfishes (*Sebastes* spp.) in the Gulf of Alaska, and Stanley et al. (1992) used the monogenean *Microcotyle sebastis* to confirm that the yellowtail rockfish *Sebastes flavidus* was distributed in discrete groups along the Pacific coast of North America. However, there is not much information on *Sebastes* spp. from the Northwest Atlantic because studies of redfish parasites prior to that of Bourgeois and Ni (1984) must be treated with caution because of the possible confusion in identification of redfish species (Moran et al., 1996).

Materials and methods

Host and parasite collections

A total of 170 deepwater redfish of size >22 cm were sampled by bottom trawl from five areas representing four management units off the Atlantic coast of Canada between August 1996 and January 1997. Summer and winter samples were available only in unit 2 (Fig. 1, Table 1). Fish were measured on board, individually bagged, and deep frozen immediately after capture for later examination. Because fish from southwestern Labrador Sea and Flemish Cap were not measured on board, estimates of fresh length were made for these fish by using a regression of fresh length on frozen length obtained from the other fishes.

Fish were thawed in the laboratory, measured (total length), and weighed. Prior to parasitological examination, species of redfish were identified by the number of soft anal-fin rays. Only those that had 8 or more soft rays at the anal fin were retained for the analyses (Ni, 1981). Examinations for metazoan parasites were performed with a stereomicroscope using standard parasitological methods. The external surface was examined and scars from previous infestations of *S. lumpi* were noted. The gills were removed, rinsed, and their arches were detached and examined separately. Because of the pressure changes when redfish were hauled out from the deep water most of the stomachs were everted. The internal organs (heart, liver, spleen, gall bladder, swim bladder, digestive tract, gonads, kidney, urinary bladder) were inspected for parasites (lying free or encapsulated on the exterior),

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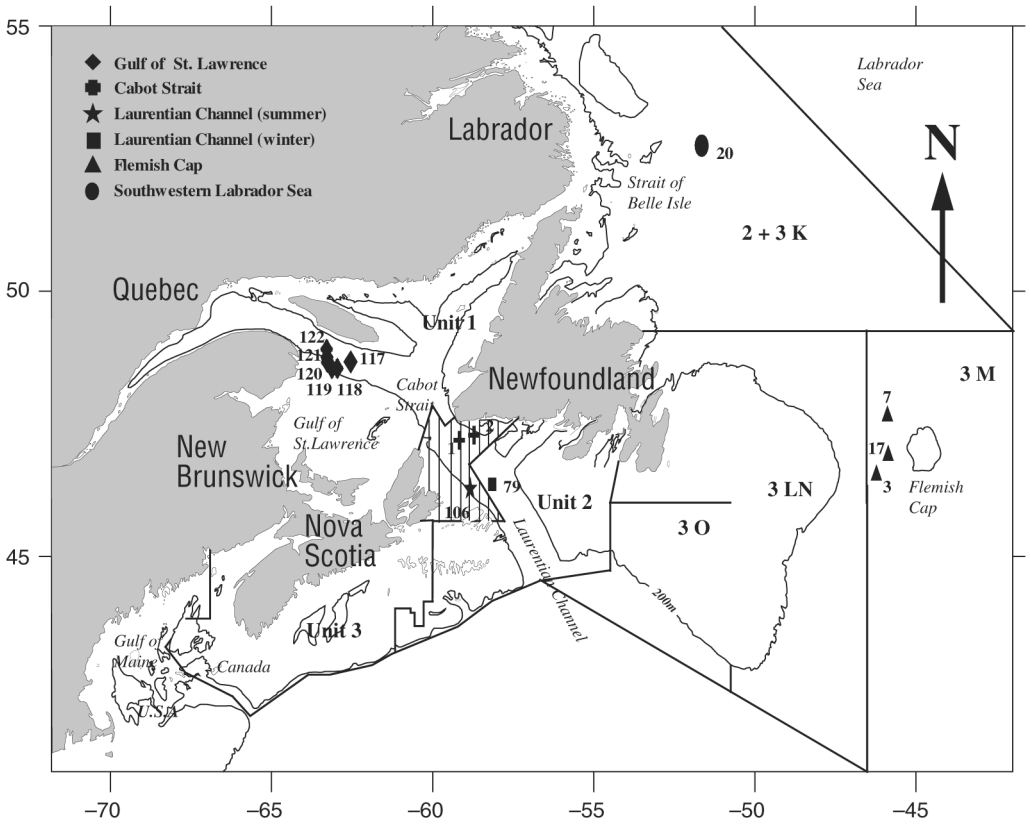

Figure 1

Map of redfish (*Sebastes mentella*) collection sites (various symbols) in the northwestern Atlantic Ocean in 1996 and 1997. The eight areas currently used for redfish management are delineated: 1) subarea 2 and division 3K (southwestern Labrador Sea), 2) divisions 3LN (northern and western Grand Banks); 3) division 3M (Flemish Cap); 4) division 3O (southwestern Grand Banks); 5) unit 1 (Gulf of St. Lawrence); 6) unit 2 (Laurentian Channel); 7) unit 3 (Scotian Shelf); and 8) the Gulf of Maine. The hatched area (Cabot Strait) is part of unit 1 from January to May and unit 2 from June to December. Numbers near collection sites represent set numbers. Jagged line indicates the 200-m depth contour.

separated, and then examined individually. The stomach, pyloric ceaca, and intestine were separated, opened longitudinally, and their contents rinsed into beakers where they were mixed with sodium bicarbonate and allowed to settle to remove endoparasitic helminths. The wall of the stomach, pyloric ceaca, and intestine, and the liver, spleen, kidney, and heart were compressed between glass plates and examined for parasites. The body musculature was removed from the vertebral column, the skin was removed from the fillets, and flaps were thinly sliced and all were inspected for helminths and dead neck stalks or sores caused by old infestations by *Sphyrion lumpi*.

All parasites were sorted into major taxonomic groups, cleaned, and counted for each organ. Nematoda and Copepoda were identified fresh to the lowest taxon and then fixed in 70% ethanol with 10% glycerin. Old cephalothoraces and sores caused by *Sphyrion lumpi* were identified and counted. Digenea and Cestoda were fixed in alcohol-formalin-acetic acid (AFA) and stained in acetocarmine for later identification.

Statistical analyses

Results of analyses may be influenced by variations in factors such as size and sex. In our study, there were no significant differences in mean size of fish sampled from the various regions (Table 1). Males were smaller than females from all regions, except those from the Labrador Sea, where the size difference was reversed. However, none of the parasites retained for multiple parametric analyses differed between the sexes (ANOVA, $P>0.05$).

Because parasite counts for all areas were not normally distributed and normality could not be reached by using various transformations, multiple nonparametric analyses (SAS version 8.0, SAS Institute, Inc., 1999) were used to investigate the usefulness of parasites in discriminating host collections. All parasites used in this analysis were relatively long lived and thus accumulated with host age (length). However, the relationship between size and intensity of certain parasites (*Anisakis simplex*) was not linear, thus rendering covariant analyses inappropriate.

Table 1

Summary of collection data for redfish (*Sebastes mentella*) in the Northwest Atlantic Ocean, 1996–97.

Geographic region	NAFO management area	Latitude-longitude	Date	Number of fish	Mean length (mm) ±SD (range)
Gulf of St. Lawrence	unit 1 (4T)	48.91°N, 63.29°W to 48.66°N, 62.55°W	August 1966	30	330.0±37.4 (220–400)
Cabot Strait	unit 2 (3Pn)	47.19°N, 59.18°W to 47.26°N, 58.72°W	August 1996	49	318.0 ±30.5 (220–390)
Laurentian Channel	unit 2 (4Vn)	46.28°N, 58.84°W	August 1996	31	315.2 ±22.3 (250–350)
Southwestern Labrador Sea	2J	52.74°N, 51.65°W	Autumn 1996	13	321.5 ±59.6 (250–440)
Flemish Cap	3M	47.64°N, 45.89°W to 48.08°N, 44.55°W	Autumn 1996	16	317.0 ±53.6 (228–443)
Laurentian Channel	unit 2 (3Ps)	46.36°N, 58.15°W	January 1997	31	324.5 ±27.5 (260–390)

Table 2

Number of fish involved in redfish (*Sebastes mentella*) statistical comparisons of parasite mean abundance between management units. Winter and summer samples from unit 2 were pooled for these analyses.

	Length class (mm)														
Comparison	220	260	280	290	300	310	320	330	340	350	360	380	390	440	All
FlemishCap—Labrador Sea		2	2	2	2	3	3		3		3			2	22
Flemish Cap—unit 2		3	7	5	12	24	25		14		4	4			98
Labrador Sea—unit 1				2	3		8	5	4		5				27
unit 1 and unit 2	2			5	13		30	18	15	10	6		3		102

The following analyses were performed. For each pair of geographically adjacent management units, that is, across the four stock boundaries encountered around Newfoundland (See Fig. 1), fish were divided into 1-cm length classes. For a given comparison, only length classes containing fish from both units involved were retained. Within each class, the parasite infection intensities were attributed standard normal ranks. The ranks from all length classes were then combined to perform a *t*-test. This test corrects for the effects of host size on parasite mean abundance and for variance heterogeneity while preventing outlying observations from having too much influence. The multiplicity of the statistical tests (many pairs of stocks multiplied by many parasites) was accounted for by a bootstrap procedure (PROC MULTTEST, SAS, version 8, SAS Institute, Inc., 1999). The numbers of fish from each length class involved in the comparisons are detailed in Table 2.

Intensity refers to the number of parasites of a given species in an infected individual fish and mean intensity refers to the mean number of parasites of a given species per infected fish in a sample. Mean abundance is defined as the mean number of parasites of a given species per host, infected and uninfected, in a sample. Prevalence is the proportion of fish infected with a given parasite in a sample, expressed as a percentage (Bush et al., 1997).

Results

Sixteen taxa were found to infect *S. mentella* in eastern Canada in this survey. These included one myxozoan (*Ceratomyxa* sp.), eight digeneans (*Anomalotrema koiae*, *Derogenes varicus*, *Hemiuris levinseni*, *Lecithaster gibbosus*, *Lecithophyllum botryophorum*, *Olssonium turneri*, *Podocotyle reflexa*, and *Progonus muelleri*), two cestodes (*Bothriocephalus scorpii*, and *Scolex pleuronectis* plerocercoids), three nematodes (*Anisakis simplex*, Contracaecinea, and *Hysterothylacium aduncum*), and two copepods (*Chondracanthus nodosus* and *Sphyrion lumpi*). This is the first report of *O. turneri* and *P. muelleri* from *Sebastes* spp.

Preliminary analyses demonstrated that only larvae of *Anisakis simplex* and *Hysterothylacium aduncum*, both anisakid nematodes occurring on the viscera of fish, and the copepod ectoparasite *Sphyrion lumpi* could be used

Table 3

Prevalence (%), mean intensity (no. of parasites, ±SD), and range of intensity of infections of redfish (*Sebastes mentella*) with parasites used as biological tags in the Northwest Atlantic. *n* = sample size.

Region	*n*	Parasite	Prevalence (%)	Intensity (mean ±SD)	Range of intensity
Gulf of St. Lawrence (unit 1-4T)	30	*Anisakis simplex*	53.3	2.8 ±3.1	1–13
		Hysterothylacium aduncum	20.0	1.2 ±0.4	1–2
		Sphyrion lumpi	23.3	1.7 ±0.8	1–3
Cabot Strait (unit 2-3Pn)	49	*Anisakis simplex*	26.5	2.0±1.2	1–4
		Hysterothylacium aduncum	44.9	1.6 ±0.7	1–3
		Sphyrion lumpi	51.0	1.9 ±1.4	1–7
Laurentian Channel–summer (unit 2–4Vn)	31	*Anisakis simplex*	16.1	1.0 ±0.0	1
		Hysterothylacium aduncum	32.3	1.5 ±1.0	1–4
		Sphyrion lumpi	67.7	1.7 ±0.9	1–4
Laurentian Channel–winter (unit 2–3Ps)	31	*Anisakis simplex*	29.0	1.1±0.3	1–2
		Hysterothylacium aduncum	29.0	1.4 ±0.7	1–3
		Sphyrion lumpi	41.9	2.2 ±2.2	1–9
Labrador Sea (2J)	13	*Anisakis simplex*	46.2	25.2 ±35.5	1–92
		Hysterothylacium aduncum	30.8	7.0 ±6.8	2–17
		Sphyrion lumpi	7.7	2.0	2
Flemish Cap (3M)	16	*Anisakis simplex*	56.3	2.7 ±3.5	1–12
		Hysterothylacium aduncum	93.8	93.8 ±92.4	1–259
		Sphyrion lumpi	6.3	10.0	10

to discriminate among redfish stocks (SAS GLM procedure, SAS, 2001). Gastrointestinal digeneans were not used because differences were not noted among regions (SAS GLM procedure). Furthermore, evagination of most of the redfish stomachs during collection caused losses of gastrointestinal parasites and rendered the worm counts unreliable. Myxozoans and *C. nodosus* were not common enough to be used as tags for deepwater redfish (1% and 2% prevalence overall, respectively). *Sphyrion lumpi* use redfish as its definitive host but only adult females are embedded permanently in the flesh, and sores of previous infections were taken into account in our study.

Larvae of *A. simplex* were common at almost all sites, but were particularly abundant off Labrador, on the Flemish Cap, and in the Gulf of St. Lawrence. The most prevalent and abundant parasite encountered was *H. aduncum* in redfish from the Flemish Cap. The copepod *S. lumpi* was most prevalent in the Gulf of St. Lawrence and the Laurentian Channel. Prevalence and mean intensity of these parasites are shown in Table 3.

Results of multiple nonparametric analyses demonstrated that at least one of the three parasite species differed in mean abundance between all adjacent areas, with the exception of the Labrador Sea and the Gulf of St. Lawrence. Mean abundance of both *H. aduncum* and *S. lumpi* in redfish from the Flemish Cap and the Cabot Strait–Laurentian Channel were significantly different (bootstrap adjusted $P<0.0001$ and 0.01, respectively). Mean abundance of *H. aduncum* also differed between fish from the Flemish Cap and the Labrador Sea (bootstrap adjusted $P<0.05$). Lastly, mean abundance of *A. simplex* differed between fish from the Gulf of St. Lawrence and those from the Cabot Strait–Laurentian Channel (bootstrap adjusted $P<0.01$) (Table 4).

Given that the Gulf of St. Lawrence (unit 1) and the Cabot Strait–Laurentian Channel (unit 2) populations are currently managed as separate stocks, we wished to validate our results. Analyses demonstrate that for *A. simplex*, mean abundance is significantly different in fish from unit 2 collected in winter and those from the Gulf of St. Lawrence (unit 1) collected in summer (bootstrap adjusted $P<0.05$), but no difference could be shown between those collected in winter versus summer from unit 2, nor between those collected in the summer compared to winter within the Cabot Strait–Laurentian Channel (Table 5).

Discussion

Criteria for the use of parasites as biological markers of fish populations have been reviewed by Williams et al. (1992). In this study, we employed the following four criteria for biological markers: geographic variation in prevalence or abundance, longevity of infection, absence of reproduction directly in or on the host, and ease of detection and enumeration. First, geographic variation in prevalence and abundance was observed in the various parasites. Second, the parasites or their remains have a

Table 4

Nonparametric length-stratified comparison of parasite mean abundance in redfish (*Sebastes mentella*) between management units. Winter and summer samples from unit 2 were pooled for these analyses. Overall test multiplicity is corrected for by bootstrap.

Parasite	Comparison	Raw *P*-value	Bootstrap *P*-value
Anisakis simplex	Flemish Cap and Labrador Sea	0.4941	0.9997
	Flemish Cap and unit 2	0.0083	0.0900
	Labrador Sea and unit 1	0.0184	0.1932
	unit 1 and unit 2	0.0008	0.0090*
Hysterothylacium aduncum	Flemish Cap and Labrador Sea	0.0014	0.0151*
	Flemish Cap and unit 2	<.0001	<0.0001*
	Labrador Sea and unit 1	0.1794	0.9039
	unit 1 and unit 2	0.1546	0.8629
Sphyrion lumpi	Flemish Cap and Labrador Sea	0.7774	1.0000
	Flemish Cap and unit 2	0.0006	0.0077*
	Labrador Sea and unit 1	0.3077	0.9872
	unit 1 and unit 2	0.0308	0.3065

Table 5

Nonparametric, length stratified comparison of parasite mean abundance in redfish (*Sebastes mentella*) between unit 1 (Gulf of St. Lawrence) and unit 2 (Cabot Strait–Laurentian Channel). Overall test multiplicity is corrected for by bootstrap.

Parasite	Comparison	Raw *P*-value	Bootstrap *P*-value
Anisakis simplex	unit 1(summer) and unit 2 (winter)	0.0042	0.0238*
	unit 2 (summer) and unit 2 (winter)	0.9150	1.0000
Hysterothylacium aduncum	unit 1(summer) and unit 2 (winter)	0.9050	1.0000
	unit 2 (summer) and unit 2 (winter)	0.1613	0.6454
Sphyrion lumpi	unit 1(summer) and unit 2 (winter)	0.4326	0.9653
	unit 2 (summer) and tunit 2 (winter)	0.4018	0.9517

long life span in their hosts. Larval anisakid nematodes can survive for a number of years in the fish host; McClelland and Marcogliese (1994) provided a detailed discussion of the use of anisakid parasites as biological tags for fish stocks. They pointed out that although prevalence and abundance of anisakids may vary over longer time scales, they are stable over the time frame (a few years) of a given survey, rendering them suitable for a stock discrimination study. Remarkably, of the 24 studies on biological tags reviewed by Arthur (1997) between 1990 and 1997, eight of them employed anisakid nematodes. Although the copepod *S. lumpi* has a limited life span, the cepahlothorax and scar tissue from previous infections persist for many years (Templeman and Squires, 1960; Sindermann, 1961; Reimer and Szuks, 1989; Bakay, 1988[1]). Third, none of these parasites multiply directly on the host and thus augment their abundance, as in the case of some protozoans and monogeneans. The anisakids are larval stages, and the copepod produces free-swimming larvae. Lastly, the parasites chosen are easily detected and counted.

We have shown that the parasite fauna of the deepwater redfish (*S. mentella*) can provide useful information on stock discrimination and definition of stock boundaries, as has been demonstrated for other fishes (see Williams et al. 1992; Arthur, 1997). Indeed, fish from all adjacent zones, with the exception of the Labrador Sea and Gulf of St. Lawrence, could be separated on the basis of abundance of at least one species of parasite. Distinction between redfish from the Gulf of St. Lawrence (unit 1) and the Cabot Strait–Laurentian Channel (unit 2) is reinforced by analyses demonstrating differences between the mean abundance of *A. simplex* in fish collected from the Gulf in summer and those in unit 2 in winter or summer. Furthermore, there were no differences in abundance of *A. simplex* detected in fish from unit 2 between winter and summer. Our results also support those of previous studies that demonstrated differences between redfish from the Flem-

[1] Bakay, Y. I. 1988. Application of results from parasitological investigations in redfish (*Sebastes mentella* Travin) populational structure studies. Int. Coun. Explor. Sea C.M. 1988/G: 35, 14 p.

ish Cap and the Labrador Sea according to their parasite fauna (Templeman and Squires, 1960; Bourgeois and Ni, 1984). All areas sampled in our study are currently managed as separate stocks, and our results do not suggest that management strategies should change. However, the inclusion of Cabot Strait in unit 1 in the winter should be re-evaluated with further sampling.

Our parasite results are in contrast with results using microsatellite DNA markers (Roques et al., 2002), in which *S. mentella* from the Gulf of St. Lawrence could not be differentiated from those of the Laurentian Channel. For this species, only three divergent populations were defined across the North Atlantic: 1) a western population in the Gulf of St. Lawrence and offshore Newfoundland; 2) a panoceanic population; and 3) an eastern population in Norway and the Barents Sea. Thus redfish from unit 1 could not be differentiated from those of unit 2. Similar results were reported for *S. fasciatus* sampled in units 1 and 2 (Roques et al., 2001). Redfish populations in both units are characterized by the presence of hybrid and introgressed individuals between *S. fasciatus* and *S. mentella* (Roques et al., 2001). Stock distinction does not preclude genetic exchange between stocks, but managers must be aware of the size and spatial boundaries of stocks, as well as the level of gene flow between the stocks.

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