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Putative histiocytic sarcoma in redfin needlefish *Strongylura notata* (Beloniformes: Belonidae) in Florida, USA

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ABSTRACT: Redfin needlefish *Strongylura notata* from Florida coastal waters were observed with unusual neoplastic lesions. Affected specimens were collected from 1 Atlantic estuary (Indian River Lagoon, prevalence = 0.32%, n = 5314) and 2 Gulf of Mexico estuaries (Tampa Bay, prevalence = 0.02%, n = 10762; Charlotte Harbor, prevalence = 0.02%, n = 5112) during routine fisheries-independent monitoring surveys conducted from 1999–2009. Grossly, each lesion manifested as a large (18–30 mm × 20–50 mm), raised (approximately 10 mm), white, creamy, or pinkish nodule on the flank, dorsal trunk, base of the pectoral fin, or head. Multiple small (<5 mm) nodules possessing poorly demarcated borders with neighboring tissues on the external jaw surface and at the base of the teeth were also observed. Histopathologically, neoplastic cells were found in the dermis, beneath the skeletal muscle, and in the soft tissue at the base of teeth of the premaxilla and the dentary jaw processes. Neoplastic cells usually had prominently invaded among the myosepta of the skeletal muscle. Neoplastic parenchymal cells had the basic characteristics of atypical, mononuclear, round, histiocytic cells with an eccentric, reniform nucleus and abundant cytoplasmic vacuolation, while some exhibited bizarre nuclear pleomorphism. Transmission electron microscopy revealed that neoplastic cells had a grooved nucleus and cytoplasmic organelles with rough endoplasmic reticulum, mitochondria, Golgi apparatus, and lysosomes. Neoplastic cells had possibly metastasized to liver, spleen, and kidney. Positive immunohistochemical staining with Ki67, p53, S-100, and CD163 support neoplastic features and a putative diagnosis of histiocytic sarcoma.

KEY WORDS: Histiocytic sarcoma · Redfin needlefish · Hematopoietic neoplasm · Tumor · Florida · Indian River Lagoon · Immunohistochemistry · Transmission electron microscopy

1. INTRODUCTION

In Florida (USA), the redfin needlefish *Strongylura notata* is most abundant along the central and southern regions of the Gulf and Atlantic coasts, where it is commonly sampled by the Florida Fish and Wildlife Conservation Commission's (FWC) Fish and Wildlife Research Institute (FWRI) during routine fisheries-independent monitoring (FIM) surveys (FWC-FWRI 2012). The redfin needlefish is a small to medium-size (usually <380 mm but up to 610 mm total length), short-lived (seldom exceeding 3 yr), shallow-water predator (Robins & Ray 1986), and 0 to 2 yr old individuals are numerically dominant in Florida populations (Breder 1932). The species is used as bait by anglers targeting large sport fish such as marlins *Makaira* spp. (Collette 2002). The fish population surveys are conducted year-round in 6 of the state's major estuarine systems (including the Indian River Lagoon, Charlotte Harbor, Tampa Bay, Cedar Key,

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Apalachicola Bay, and the Saint Johns River), and standardized, stratified random sampling methods are used that explicitly include collection and documentation of fish exhibiting grossly obvious lesions (FWC-FWRI 2013).

During surveys, redfin needlefish having grossly visible external nodular lesions on the skin and jaw tissues were readily confirmed, although the likelihood of encountering affected fish was extremely low. Initial histopathological features revealed that the nodular lesions appeared to be a type of hematopoietic neoplasm. Characterization of this neoplastic lesion in redfin needlefish as described herein, and based on morphological and immunohistochemical properties, putatively suggests it is a histiocytic sarcoma (HS).

As malignant tumors of histiocytes, HS is rarely reported in humans (Pileri et al. 2002, Hornick et al. 2004, Takahashi & Nakamura 2013), more often in domestic animals (Fulmer & Mauldin 2007, Moore 2014, Ogihara et al. 2016), and occasionally in captive mammals and birds (Sacre et al. 1992, Molenaar et al. 2009, Canuti et al. 2014). In teleosts, closely-related hematopoietic neoplasia (lymphosarcoma, lymphoma) have been reported in multiple species (Nigrelli 1947, Sonstegard 1975, Bowser et al. 1985, 2002, Békési & Kovács-Gayer 1986, Hoffmann et al. 1988, Okihiro & Hinton 1989), and although apparently rare (or underreported) in wild aquatic animals, HS has been described in 1 species of freshwater fish, the northern pike *Esox lucius* (Thompson & Kostiala 1990). In human and veterinary medicine, verification of a histiocytic lineage using specific immunohistochemical markers as well as morphological characteristics are useful in HS diagnosis (Pileri et al. 2002, Hornick et al. 2004, Vos et al. 2005, Takahashi & Nakamura 2013, Ansari et al. 2016).

The low prevalence of putative HS in Florida redfin needlefish had obscured its potential significance as an indicator of fish and environmental health until temporal and spatial analyses of long-term FWC data revealed significant spatial differences in prevalence among the 6 sampled Florida estuaries within the geographic range of redfin needlefish. These data suggest the potential for a multifactorial etiology with unknown cofactors that may be present at relatively higher incidence in those estuaries presenting with neoplastic fish. Here, we used 11 yr of FIM data to report the prevalence and distribution of characteristic neoplastic lesions in redfin needlefish from Florida estuaries. In support of the diagnosis, we provide gross, histopathological, fine ultrastructural, and immunohistochemical descriptions of the HS lesions, compared to closely related tumors in other species.

2. MATERIALS AND METHODS

2.1. Fish collection

We began this investigation after recognizing that a recurring, morphologically distinct, neoplastic lesion was present in a few specimens of redfin needlefish collected by FIM during routine surveys. To investigate trends in prevalence and describe the lesion, we evaluated historical data sets, examined and processed archived tissue samples, and reviewed photographic documentation. Our evaluation primarily focused on data and materials collected from January 1999 through December 2009, although here we reference data from monitoring conducted through December 2016.

Stratified random sampling was conducted monthly in each estuary (see site description below). Fish were captured with one of the following gear types: (1) a 21.3 m center-bag haul seine made of 3.2 mm nylon mesh, (2) a 183 m center-bag haul seine made of 38 mm stretch nylon mesh, or (3) a 61 m center-bag seine made of 25 mm stretch nylon mesh. Nets were deployed along the shoreline, and the 21.3 m seine was also used away from the shoreline along shallow flats in water to a maximum depth of 1.8 m. Sampling methods were standardized among estuaries and from year to year except that the 21.3 m seine was not used in the southern Indian River Lagoon.

In the field, fish were counted and measured (standard length [SL]; mm), and all specimens ≥75 mm SL were evaluated for gross physical abnormalities. Fish with visible lesions were iced immediately in the field. Specimens collected from the Tampa Bay region were returned to the FWRI St. Petersburg laboratory for necropsy and processed either on the day of capture or the following day. Specimens collected in other regions were either fixed with 10% neutral buffered formalin at the end of the sampling day or kept on ice and shipped overnight to St. Petersburg for processing.

2.2. Site description

Fish were sampled in 6 Florida estuaries including tidally influenced portions of rivers (Fig. 1): (1) Indian River Lagoon (IRL, including the Indian, Banana, and St. Sebastian Rivers but artificially excluding the Mosquito Lagoon, where sampling coverage was inadequate for consideration here), (2) Charlotte Harbor and Pine Island Sound (CH), (3) Tampa Bay (TB, including the Alafia, Braden, and Little Manatee

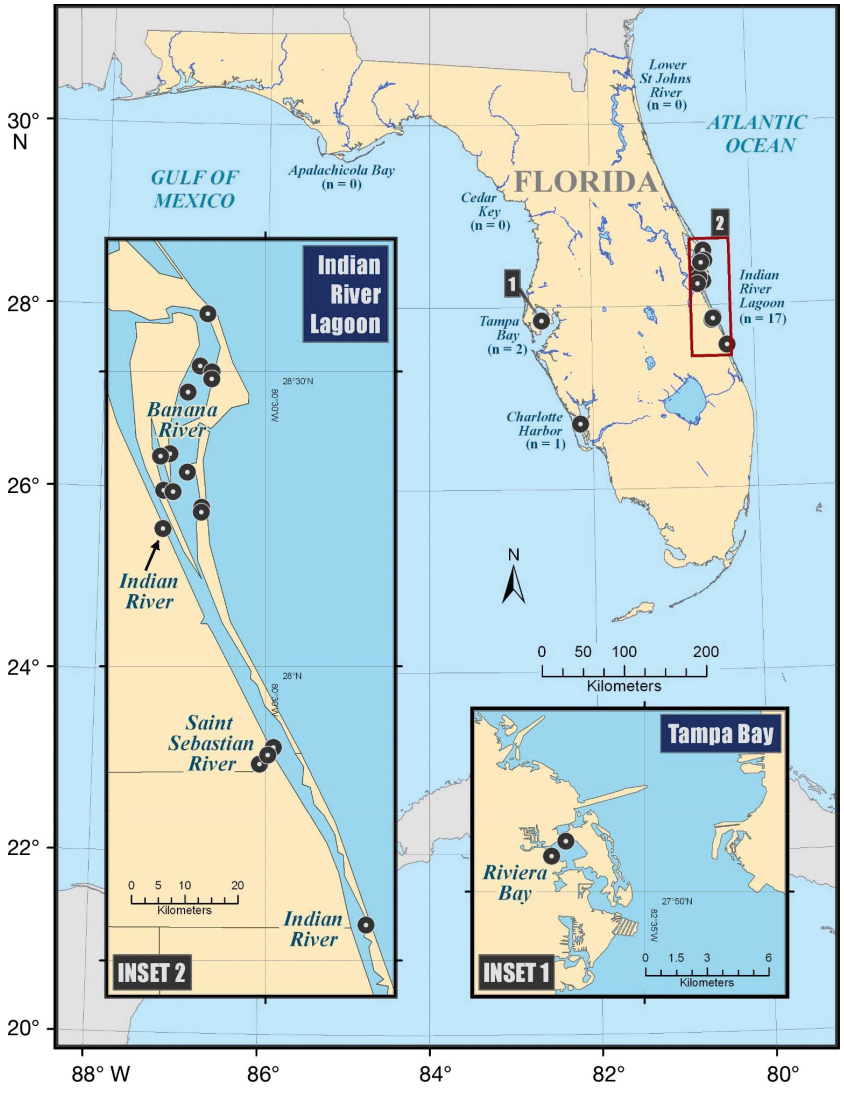

Fig. 1. Sites of sampled estuaries: Indian River Lagoon, Charlotte Harbor, Tampa Bay, Cedar Key, Apalachicola Bay, and Lower St. Johns River, Florida, USA. Insets 1 (Tampa Bay) and 2 (Indian River Lagoon) are at larger scales, with each positive neoplasm case in redfin needlefish shown as a dotted circle

Table 1. Number, prevalence (%, in parentheses) and anatomical locations of external neoplastic nodular lesions of redfin needlefish examined in the Indian River Lagoon (IRL), Tampa Bay (TB), and Charlotte Harbor (CH), Florida, USA, during 1999–2009; n: total number of fish

Anatomical location of neoplastic nodules	IRL (n = 5314)	TB (n = 10 762)	CH (n = 5112)
Jaw	4 (0.08)	–	1 (0.02)
Flank	8 (0.15)	1 (0.01)	–
Trunk dorsal	3 (0.05)	–	–
Pectoral fin base	1 (0.02)	1 (0.01)	–
Head ventral	1 (0.02)	–	–
Total neoplastic nodules	17 (0.32)	2 (0.02)	1 (0.02)

Rivers), (4) vicinity of Cedar Key (CK, including Suwannee Sound, Waccasassa Bay, and the Suwannee River), (5) Apalachicola Bay, St. George Sound, and the Apalachicola River (AB), and (6) lower (northern) St. Johns River (SJR).

2.3. Gross observations

Fresh specimens that exhibited lesions were examined as soon as possible to minimize changes post-mortem. Gross examinations were conducted to descriptively and photographically document the neoplastic lesion for anatomical location (Table 1), size, shape, color, cut appearance, and consistency.

2.4. Histopathology

Specimens collected during 1999 through 2009 from the IRL (cases 1–12), TB (case 14), and CH (case 15), and fixed within 24 h after capture were used for descriptive purposes to minimize misinterpretation of post-mortem fixation artefacts (Table 2). For all specimens, we evaluated affected skin and muscle tissue, and where possible, internal tissues: liver (n = 4), spleen (n = 3), and posterior kidney (n = 3). Additionally, tissues (jaw, liver, spleen, middle to posterior kidney, and heart) from a lesioned specimen freshly caught in February 2015 from the IRL (case 13; Table 2) were examined and fixed within 6 h after capture. These tissues were suitable for histopathological, immunohistochemical, and ultrastructural studies.

Small pieces of grossly appearing neoplastic tissue including the skin and underlying skeletal muscle or jaw, and surrounding clinically healthy tissues, were fixed in 5% paraformaldehyde or 10% neutral buffered formalin and then decalcified with formic acid except for the 2 samples which were not decalcified but were used for immunohistochemistry studies (see below, cases 12 and 13). Tissues were embedded in paraffin or JB-4 resin, or in both media. Sectioned slides 4 µm thick were stained routinely

Table 2. Case numbers of redfin needlefish selected for histological, immunohistochemical, and ultrastructural examinations listed by the geographical location (Indian River Lagoon [IRL], Tampa Bay [TB], and Charlotte Harbor [CH]), chronological order of dates on which fish were collected, anatomical locations of the putative histiocytic sarcomas, and corresponding figure number(s) in the present study depicting these specimens

Case number	Geographical location	Collection date	Anatomical location of neoplasm	Figure(s)
1	IRL	9 December 1999	Trunk dorsal, kidney[a], spinal nerve tissue[a]	
2	IRL	24 September 2002	Jaw	
3	IRL	13 January 2003	Head ventral	6a
4	IRL	17 October 2005	Trunk dorsal	3b–c, 4a–b,d, 5b, 6b
5	IRL	20 March 2007	Jaw	
6	IRL	15 November 2007	Flank	
7	IRL	21 April 2008	Jaw	3d–e, 4c
8	IRL	3 September 2008	Flank	
9	IRL	24 November 2008	Flank, spleen[a]	7d–e, 9b, 10b, 11b
10	IRL	15 October 2009	Flank	
11	IRL	23 November 2009	Jaw	
12	IRL	15 December 2009	Flank	8a–b
13	IRL	12 February 2015	Jaw	5a, 9a, 10a, 11a, 12a–e
14	TB	15 February 2007	Pectoral fin base, liver[a]	3a, 7a–c, 9c, 10c, 11c
15	CH	10 May 2000	Jaw	

[a]Possible metastasis

with Mayer's hematoxylin and eosin (H&E) for paraffin embedding (Luna 1968) and with Wright's H&E, periodic acid-Schiff/metanil yellow (PAS-MY) (Quintero-Hunter et al. 1991), and thionin for JB-4 resin embedding. Light photomicrographs were taken with an Olympus BX51 microscope equipped with an Olympus DP71 digital camera.

2.5. Immunohistochemistry (IHC)

To confirm and characterize the IHC features of the neoplastic cells, 6 monoclonal or polyclonal antibodies were used: (1) Ki67 (MIB1 antibody; DAKO), cell nucleoli in late gap 1, synthesis, gap 2, and mitotic phases (Scholzen & Gerdes 2000); (2) pan keratin (AE1/AE3/PCK26 cocktail antibody; Ventana Medical Systems, product reference no. 760-2595), cells of epithelial origin (Weiss et al. 1984); (3) neuron-specific enolase (NSE antibody; Ventana Medical Systems, product reference number 760-2662), cells of neuroendocrine origin (Wick et al. 1983); (4) p53 (anti-p53 antibody [9.1], ab77813, Abcam), a tumor suppressor in various tumor types (Harris & Levine 2005, Steele & Lane 2005); (5) S-100 (polyclonal rabbit anti-S-100, Code Z0311, DAKO), macrophages, Langerhans cells, dendritic cells, glial cells (Schwann cells), and myoepithelial cells (Nakajima et al. 1982); and (6) CD163 (polyclonal rabbit anti-CD163, ab87099, Abcam), deriving from monocyte or macrophage lineages (Kristiansen et al. 2001). These antibodies were used following the manufacturers' instructions for the pre-diluted concentration (for pankeratin and NSE antibodies), or diluted 1:75 (for Ki67), 1:100 (for p53), 1:400 (for S-100), and 1:50 (for CD163) with Tris buffer saline (pH 7.6). Positive controls were human tonsil with hypertrophic lymphoid tissue (All Children's Hospital, St. Petersburg, FL; Lindboe & Torp 2002) for Ki67, p53, and CD163; human brain tissue (American Master Tech Scientific) for NSE and S-100; and pig liver (local slaughterhouse) for pankeratin. The positive control tissue reacted as expected: human tonsil tissue was Ki67-, p53-, and CD163-positive, human brain tissue was NSE- and S-100-positive, and the pig liver was pan keratin-positive.

Antibodies Ki67, pan keratin, NSE, p53, S-100, and CD163 were applied to the 2 IRL specimens with neoplastic tissues on the jaw (case 11) and flank (case 12). The latter 3 antibodies were also applied to the freshly fixed neoplastic jaw tissue (case 13), decalcified with 0.5 M ethylenediaminetetraacetic acid solution (EDTA), and to the spleen (case 9) and liver (case 14) where possible metastases occurred (see histopathology findings below; Table 2). Paraffin-embedded tissue sections (4 µm thickness) were mounted on charged slides (Surgipath), deparaffinized in xylene, and gradually rehydrated through a graded ethanol series to purified deionized water (Nanopure Infinity Ultrapure Water System, Barnstead Model D8961). Sections were placed in deionized water, placed in Declere solution containing citrate buffer (Cell Marque) for antigen retrieval, and then heat retrieved in a steamer

(Black & Decker, Model HS900) at 99.5°C for 35 min as a preparatory procedure. Endogenous alkaline phosphatases were blocked using Dual Endogenous Block (DAKO) and incubated for 10 min, followed by rinsing for 3 min with Tris-buffered saline. The sections were incubated in Nonserum Protein Block (DAKO) for 15 min. For assays using antibodies Ki67, pan keratin, and NSE, 3 serially-sectioned sets of duplicate slides from each tumor specimen were incubated in a humidity chamber (glass Petri dish) with each set exposed to 1 of the 3 antibodies at 4°C for 0.5 and 24 h, respectively. For the negative controls, another 3 sets of duplicate blank slides from each specimen were incubated identically with universal negative control (FLEX negative control, cocktail of mouse IgM, IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3; DAKO), but were not exposed to the primary antibodies. All negative control tissue slides reacted negatively. For antibodies p53 and S-100, incubation was overnight at 4°C, while for CD163, it was 30 min at room temperature. Detection of the antibody was performed with an AP-multimer (Ventana Medical Systems). The chromogen for detection was Permanent Red (DAKO). All of the slides were then counterstained with Harris hematoxylin for 30 s (Luna 1968).

2.6. Transmission electron microscopy (TEM)

For the freshly obtained specimen (case 13; Table 2), the affected area of jaw tissue was fixed in Trump's fixative (4% formaldehyde, 1% glutaraldehyde, 50 mM NaH_2PO_4, pH 7.2; McDowell & Trump 1976), followed by decalcification with 0.5 M EDTA solution overnight. The tissue was then cut to an appropriate size (approximately 1 × 1 × 1 mm) for TEM examination, and then post fixed with 1% osmium tetroxide (OsO_4) for 1 h. Tissues were dehydrated in a graded ethanol series, infiltrated with epoxy propylene oxide, and embedded in epoxy resin. The epoxy block was then sectioned with a Leica EM UC6 ultra microtome (Leica Microsystems), stained with uranyl acetate followed by lead citrate, and examined with a JEM-1400 TEM (JEOL) equipped with a digital photomicrograph ORIUS™ SC1000 CCD camera (GATAN).

2.7. Statistical analyses

Our data focused on samples collected from 1999 through 2009 because we determined that fish collected during 2010–2016 should be excluded from analyses due to drastic changes in the fish population structure in the IRL area (see details in the Discussion). Binomial analysis of variance by mixed model, or GLIMMIX (SAS Institute), was used to compare the prevalence of neoplasia among estuaries. To evaluate seasonal changes within the IRL site, categorical analyses for the presence or absence of fish with neoplasms were conducted using Fisher's exact test as at least 20% of cells had expected cell counts of <5 (SAS Institute). Seasons at the IRL site were defined following Arnold et al. (1998): spring = March–May; summer = June–August; fall = September–November; and winter = December–February.

3. RESULTS

3.1. Epizootiological findings

In total, 21 525 redfin needlefish were collected during sampling conducted by the FIM program from 1999 through 2009. The majority of these fish were captured from IRL, TB, and CH (n = 21 188), with fewer from AB, CK, and SJR (n = 337). Twenty redfin needlefish collected from the IRL (n = 17), TB (n = 2), and CH (n = 1) estuaries exhibited grossly visible, external nodular growths (Table 1). The numbers of redfin needlefish collected for IRL, TB, and CH from 2010 through 2016 were 2660, 7909, and 4250, respectively, totaling 14 819 (Table 3). During these latter 7 yr, only 1 fish collected from IRL exhibited grossly visible external nodular growth lesions.

Sample sites with affected specimens ranged, north to south, from 27.8626° N, 80.4868° W to 27.5603° N, 80.3297° W (IRL); from 27.8489° N, 82.6240° W to 27.8554° N, 82.6178° W (TB); and 26.7188° N, 82.1558° W (CH; n = 1 site only) (Fig. 1). One specimen exhibiting a nodular lesion of the jaw tissue was collected in February 2015 from the IRL, at Sebastian Inlet (27.5202° N, 80.4693° W). There were geographic differences in the prevalence of redfin needlefish with neoplasia among and within estuaries (Fig. 1). The IRL (0.32%) had a significantly higher prevalence of fish with tumors than did either TB (0.02%) or CH (0.02%) ($p < 0.0001$; Table 3). Within the IRL, of the 17 affected specimens, 12 were collected in the Banana River (50–80 km north of Sebastian Inlet), 1 each in the central and southern Indian River, and 3 near the mouth of the St. Sebastian River (Fig. 1). Of the 10 762 specimens examined from TB covering an area of 886 km^2, only 2 specimens with neoplastic lesions were collected. These 2 specimens were collected 5.5 yr apart, but within 1 km of each other in Riviera Bay (near Weedon Island, Pinellas County).

Table 3. Prevalences (%) of redfin needlefish with external neoplastic nodular growths in 3 neoplasia-positive estuarine systems (Indian River Lagoon [IRL], Tampa Bay [TB], and Charlotte Harbor [CH]) from 1999–2016. Parentheses indicate number of fish with neoplasia/total number of fish examined. Total number is sum of years excluding 2010–2016 due to a compromised fishery in the IRL areas (see 'Results' for details)

Year	IRL	TB	CH	Total
1999	0.16 (1/621)	0.00 (0/1228)	0.00 (0/295)	0.05 (1/2144)
2000	0.00 (0/579)	0.00 (0/1145)	0.19 (1/525)	0.04 (1/2249)
2001	0.50 (2/401)	0.13 (1/797)	0.00 (0/521)	0.17 (3/1719)
2002	0.38 (2/533)	0.00 (0/1046)	0.00 (0/279)	0.11 (2/1858)
2003	0.84 (2/238)	0.00 (0/887)	0.00 (0/287)	0.14 (2/1412)
2004	0.00 (0/276)	0.00 (0/833)	0.00 (0/452)	0.00 (0/1561)
2005	0.22 (1/459)	0.00 (0/994)	0.00 (0/513)	0.05 (1/1966)
2006	0.00 (0/397)	0.00 (0/643)	0.00 (0/776)	0.00 (0/1816)
2007	0.52 (3/582)	0.11 (1/897)	0.00 (0/469)	0.21 (4/1948)
2008	0.59 (3/505)	0.00 (0/1226)	0.00 (0/475)	0.14 (3/2206)
2009	0.41 (3/723)	0.00 (0/1066)	0.00 (0/520)	0.13 (3/2309)
2010	0.00 (0/164)	0.00 (0/619)	0.00 (0/735)	0.00 (0/1518)
2011	0.00 (0/93)	0.00 (0/809)	0.00 (0/384)	0.00 (0/1286)
2012	0.00 (0/230)	0.00 (0/1704)	0.00 (0/423)	0.00 (0/2357)
2013	0.00 (0/507)	0.00 (0/1438)	0.00 (0/418)	0.00 (0/2363)
2014	0.00 (0/907)	0.00 (0/1332)	0.00 (0/552)	0.00 (0/2791)
2015	0.18 (1/558)	0.00 (0/1021)	0.00 (0/556)	0.04 (1/2145)
2016	0.00 (0/201)	0.00 (0/996)	0.00 (0/1172)	0.00 (0/2359)
Total (1999–2009)	0.32 (17/5314)	0.02 (2/10762)	0.02 (1/5112)	0.09 (20/21118)

Temporal patterns in prevalence were evident both among years and seasonally. No neoplasia cases were detected in redfin needlefish in the IRL in 2000, 2004, 2006, 2010–2014, or 2016 (Table 3). No affected specimens were collected from TB during those same years, but 1 specimen with a neoplastic lesion was captured in 2000 from CH. Within the IRL, seasonal differences were observed. Prevalences of the neoplastic lesions in fall (0.60%; n = 1512), winter (0.47%; n = 845), and spring (n = 0.54%; n = 556) were significantly higher (p = 0.0038) than those in summer (0.04%; n = 2401), but there was no difference in prevalence among fall, winter, and spring. For all sites collectively, seasonal patterns in prevalence were also observed. Specimens with neoplasia were most common in fall and least common in summer, with no affected specimens collected in any estuary or year during July and August, even though the overall redfin needlefish catches were greatest during those months (Table 4).

Table 4. Monthly prevalence (number of fish with neoplasia/total number of fish examined) of redfin needlefish with external neoplastic nodular growths for all fish examined during 1999–2009 from neoplasia-positive estuarine systems (Indian River Lagoon, Tampa Bay, and Charlotte Harbor)

Month	Prevalence (%)	Neoplasia (n)	Fish (n)
Jan	0.16	2	1286
Feb	0.06	1	1680
Mar	0.16	2	1215
Apr	0.07	1	1497
May	0.07	1	1481
Jun	0.05	1	2057
Jul	0.00	0	2397
Aug	0.00	0	2309
Sep	0.16	3	1829
Oct	0.18	4	2211
Nov	0.16	3	1881
Dec	0.15	2	1345
Total	0.09	20	21 188

Mean SLs (±SE) of redfin needlefish with neoplastic lesions and of clinically healthy fish were 315 ± 11.5 mm (range 194–378 mm; n = 20) and 300 ± 0.84 mm (range 75–555 mm; n = 12 552), respectively. The length–frequency distribution was bimodal for clinically healthy fish, with modes corresponding apparently to 2 year-classes (Fig. 2). For redfin needlefish from the IRL, where most of the neoplasias were observed, the frequency of the age-0 cohort (75–225 mm SL, n = 3625) was greater than that of the age-1 cohort (275–525 mm SL, n = 1683), and all but 1 of 17 fish exhibiting neoplasia had an SL within the range of the age-1 cohort (Fig. 2). The bimodal length–frequency distribution for clinically healthy fish in TB and CH differed from that for fish in IRL, in that members of the larger cohort (age-1) were more common in catches than were smaller fish (age-0) (length–frequency distribution data not shown). The single neoplastic fish from CH and 1 of 2 from TB had an SL in the range of the age-1 cohort.

3.2. Gross observations

The neoplastic lesion usually presented as a large (18–30 × 20–50 mm, n = 8), raised (approximately 10 mm high) nodule (Fig. 3a–c, cases 14 and 4) located on the flank, dorsal trunk, base of the pectoral fin, or ventral surface of the head directly posterior to the operculum (Table 1). In some cases, multiple small (<5 mm diameter) nodules possessing poorly demarcated borders with neighboring tissues were found

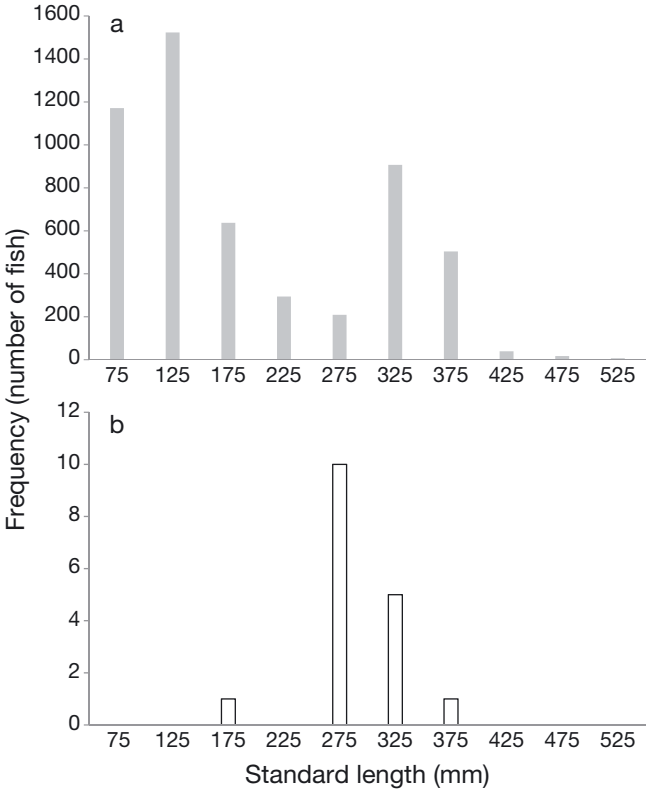

Fig. 2. Standard length–frequency distributions of redfin needlefish collected from the Indian River Lagoon that (a) were clinically healthy or (b) had neoplastic lesions

on the premaxilla and dentary jaw bones and the buccal tissue at the base of the teeth (Fig. 3d,e, case 7). Nodules had a soft consistency and were generally white, creamy white, or pinkish, sometimes with red areas.

3.3. Histopathological findings

Fourteen of the 20 specimens collected during 1999–2009, plus the 1 specimen captured in February 2015, had jaw tissue lesions with histopathological features best classified with characteristics of a hematopoietic neoplasm (Table 2). Neoplastic cells were found in the dermis (Fig. 4a, case 4), overlying the skeletal muscle (Fig. 4b, case 4), and in the soft tissue at the base of teeth in the premaxilla and dentary jaw processes (Fig. 4c, case 7). These cells prominently invaded the area among the myosepta of the skeletal muscle (Fig. 4b,d, case 4) as well as the jawbone lacunae in severely affected cases. Affected skeletal muscle tissues were associated with a floccular or granular form of fiber degeneration (granular degeneration) (Fig. 4d, case 4).

Neoplastic parenchymal cells (Fig. 5a, case 13) had the basic characteristic features of atypical round cells, with abundant, usually vacuolar, eosinophilic cytoplasm, some of which were undifferentiated. Neoplastic cells were mononuclear with an eccentric reniform or indented nucleus (Fig. 5b, case 4); presumably, these were fully mature, or they exhibited bizarre nuclear pleomorphism. A moderate number of neoplastic cells were binucleate or had 3–4 multi-lobed nuclei that resembled megakaryocytes and therefore exhibited a relatively high nuclear-cytoplasmic (N:C) volume ratio (Fig. 5b, case 4). Some neoplastic cells tended to be degenerative or necrotic, showing pyknosis, karyolysis, and in more severe cases, karyorrhexis. Mitotic figures were rarely found. No viral inclusion bodies were apparent.

As ancillary findings, neoplastic cells had commonly proliferated between stromal connective tissue populated by inflammatory infiltrates of host reactive cells, such as eosinophilic granulocytes (EGCs) (Fig. 6a, case 3). Blood vessels, especially the tunica adventitia and the tunica intermedia, also contained and were surrounded by EGCs. Neoplastic cells and newly formed capillaries replaced necrotic skeletal muscle tissues (Fig. 6b, case 4), and hemorrhages were observed. Rarely, macrophage aggregates with melanin pigments as background reactive cells were present throughout the neoplastic parenchymal cells. Sparsely distributed foci of granulomatous inflammation were detected near neighboring neoplastic cells in 1 fish.

In 3 specimens (Table 2), possible metastases were also found, along with other pathological changes, in the liver, spleen, spinal nervous tissue, and the hematopoietic tissues of the posterior kidney. The liver of 1 fish with an external neoplastic lesion showed multifocal aggregations of neoplastic cells with vital and necrotic conditions particularly surrounding or adjacent to the exocrine pancreatic tissues (Fig. 7a–c, case 14). These foci were separated from unaffected regions by stromal fibrous connective tissues adjacent to the exocrine pancreatic tissues (Fig. 7a,b) infiltrated with background reactive cells, like EGCs and macrophages. EGCs were also distributed around the neoplastic stromal connective tissues (Fig. 7b). Exocrine pancreatic tissue in the affected areas was swollen or necrotic (Fig. 7b). The liver of 1 fish (case 13) exhibited foci of alterations (preneoplastic foci) with slightly stronger basophilic appearance compared to the unaffected hepatic areas, and some parenchymal vacuolations as well. In another case (case 7), bile duct hyperplasia was found in liver tissue. In 2 specimens (cases 11 and 12), the liver was

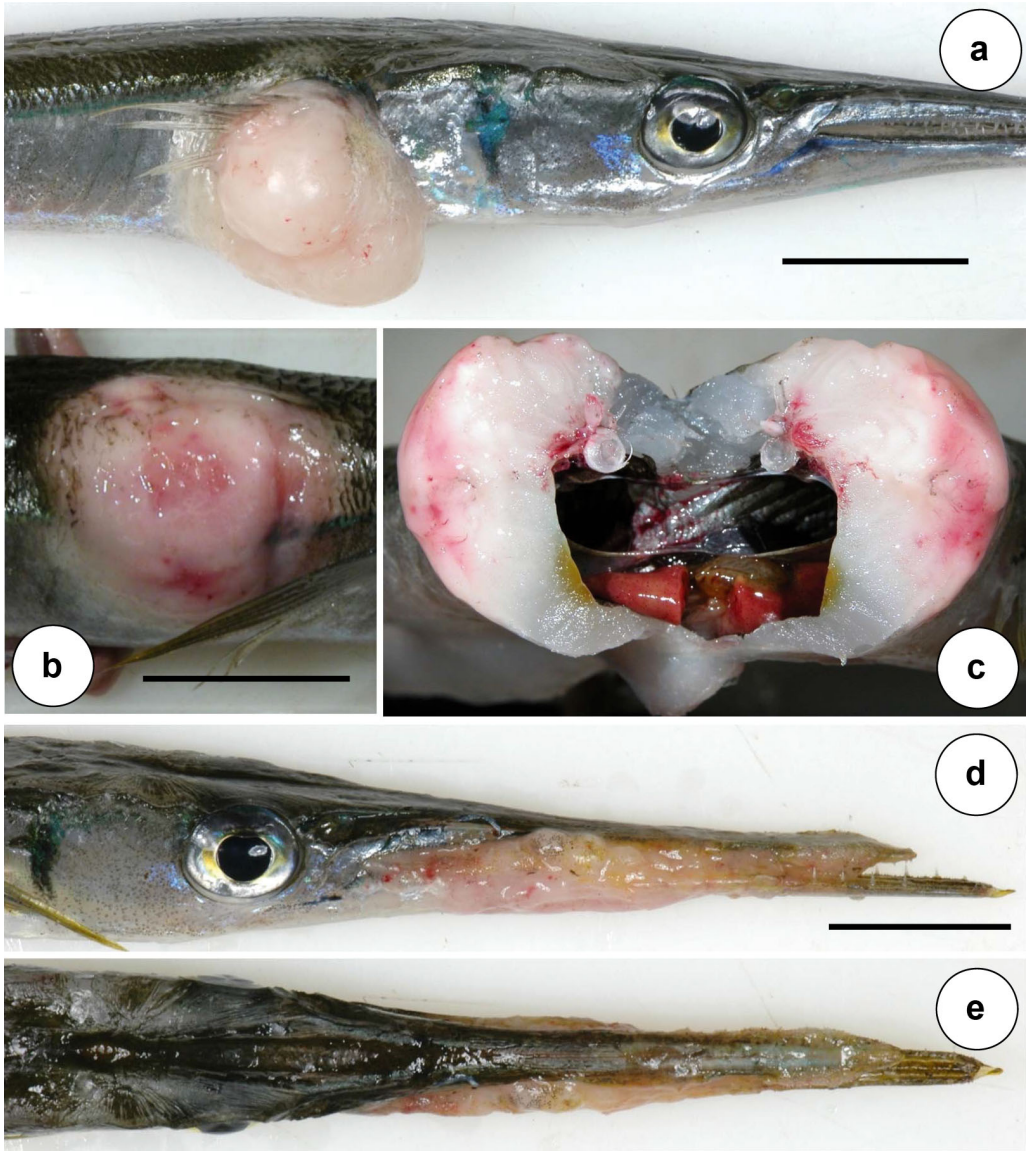

Fig. 3. Redfin needlefish specimens exhibiting gross neoplastic nodular lesion(s): (a) base of pectoral fin, right side view (scale bar = 2 cm; case 14); (b) trunk dorsal view (scale bar = 1 cm; case 4); (c) cut appearance and close-up view of (b); (d) view of right side of jaw; (e) dorsal view of jaw (d,e, scale bar = 2 cm; case 7)

necrotic. In 1 specimen, the spleen also exhibited foci (approximately 250 µm in diameter) of neoplastic cells with vital and necrotic conditions arranged in spherical masses that were encapsulated by layers of fibrous connective tissue (Fig. 7d,e, case 9). In another fish (case 7), the spleen had severe necrosis of the red pulp exhibited with karyorrhexis. Another fish (case 12) exhibited granulomatous splenitis. One specimen (case 1) with grossly observable neoplasia in the trunk dorsal muscle had neoplastic cells invading deep into the dorsal portion of the skeletal muscle, infiltrating the spinal cord and the hematopoietic tissue of the posterior kidney. The posterior kidney of another specimen (case 12) exhibited severe tubular necrosis. Incidentally, sparse, rod-shaped bacteria (approximately 7.5 µm long) were observed in the hematopoietic tissues of this specimen. Another incidental finding was a microsporidian infection in the hematopoietic tissue of the posterior kidney (case 11).

3.4. IHC

Tissues from the 2 specimens that reacted positively with Ki67 antibody had some qualitative differ-

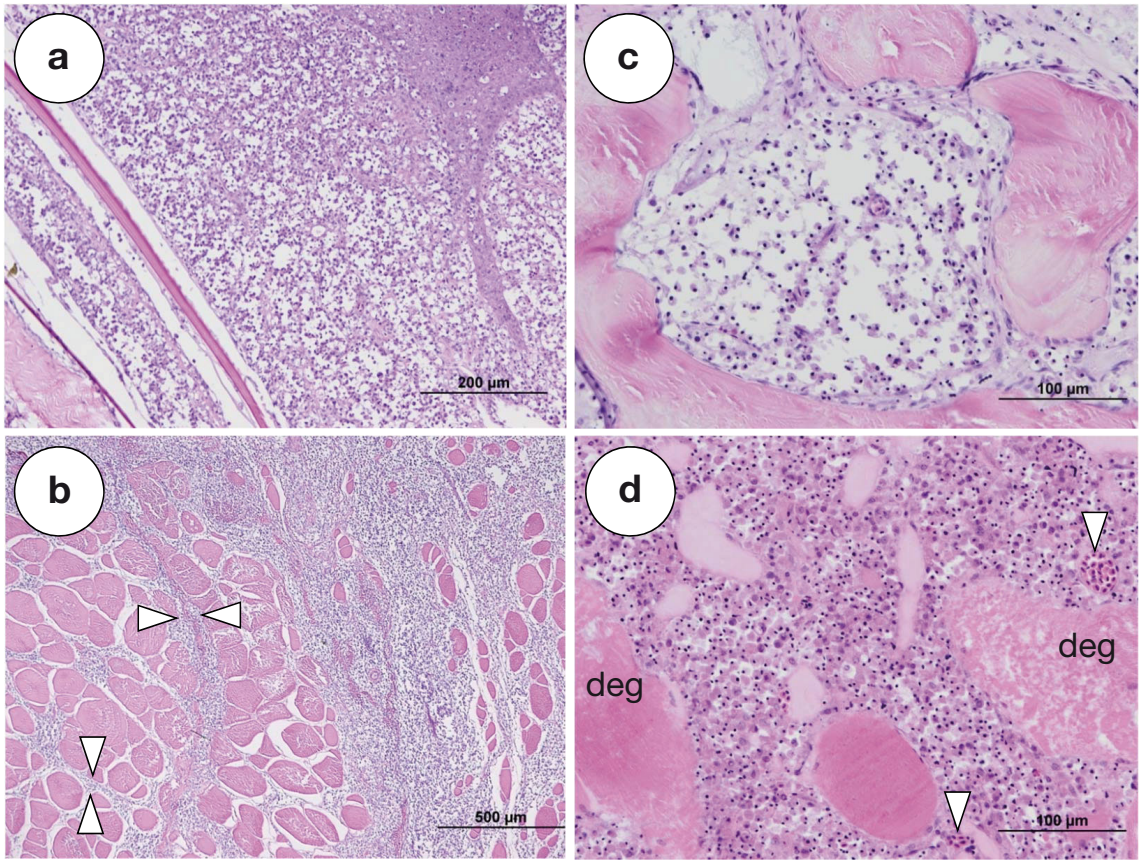

Fig. 4. Histological sections (H&E stain) of a putative histiocytic sarcoma in redfin needlefish viewed at low to moderate magnification. Neoplastic cells are invading various tissues. (a) Skin dermis (case 4); (b) deeper area of (a) among the myosepta of the skeletal muscle (closed area by 2 arrowheads); (c) jawbone (case 7); (d) skeletal muscle with degeneration (deg) and vascularization (arrowheads) (case 4)

ences. In the first specimen (case 12), neoplastic parenchymal cells in the skin and underlying skeletal muscle tissue exhibited a positive reaction in multifocal areas after 0.5 h of antibody incubation (Fig. 8a). After 24 h of incubation, the Ki67 reaction was much stronger, with diffuse positive staining in all apparently affected parenchymal cells (Fig. 8b). Remnants of skeletal muscle fibers, blood vessels, and luminal erythrocytes exhibited a negative Ki67 reaction. In the second specimen (case 11), the neoplastic cells in the jaw tissue did not react following 0.5 h of incubation, but they exhibited a light positive reaction (i.e. positive cells were randomly distributed) in the parenchyma after 24 h of incubation (Table 5).

A positive reaction to p53 (Fig. 9), S-100 (Fig. 10), and CD163 (Fig. 11) antibodies was found in 3 tissues with invading neoplastic cells, in flank (case 12) and jaw (cases 11 and 13; case 13 is shown in Figs. 9a, 10a, & 11a), as well as spleen foci (case 9, Figs. 9b, 10b, & 11b), and liver foci (case 14, Figs. 9c, 10c, & 11c) of the fish that had an external neoplastic lesion (Table 5).

Neoplastic parenchymal cells in cases 11 and 12 did not react to pan keratin for either 0.5 or 24 h incubation times, while the areas surrounding the blood vessels and the endothelial cell linings stained positive. Also, these cases did not react to NSE after 0.5 or 24 h of antibody incubation (Table 5), but in case 12 (skin and skeletal muscle tissue), a positive reaction was observed in the surrounding blood vessels, which had a stringy, fibrous appearance.

3.5. TEM

The HS occurring in the jaw of redfin needlefish putatively diagnosed by light microscopy (LM) was further described ultrastructurally (case 13 only). A typical neoplastic cell had an eccentric nucleus and abundant perinuclear cytoplasmic vacuolation (Fig. 12a–d). Some nuclei, however, exhibited groove-like structures, or indentations, or were invaginated (Fig. 12e). The nucleus typically possessed nucleoli

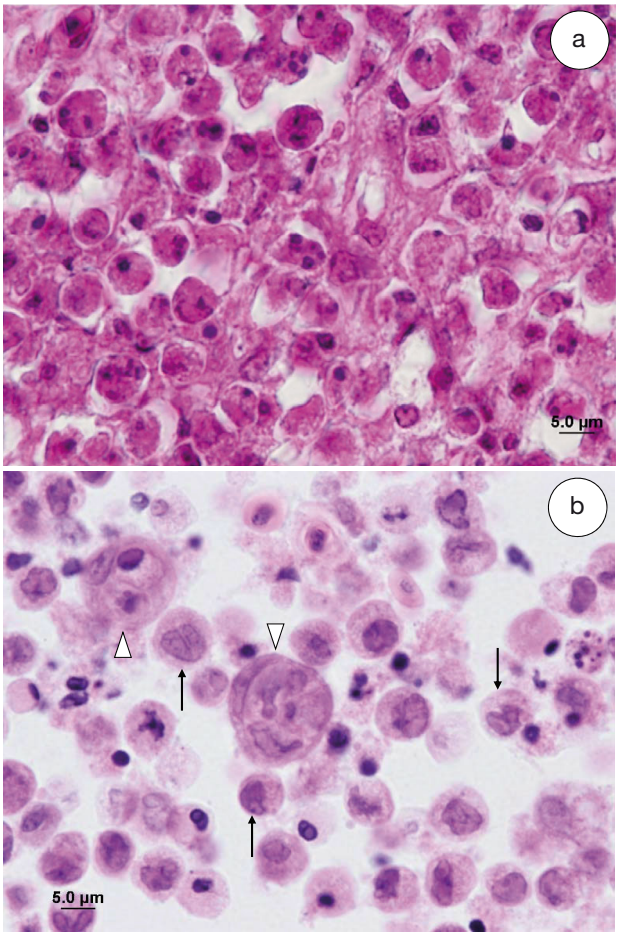

Fig. 5. Histological sections (H&E stain) of a putative histiocytic sarcoma in redfin needlefish viewed at high magnification. Characteristic features of the neoplasia are shown. (a) Neoplastic cells with features resembling atypical mononuclear round cells in the jaw tissue (case 13). (b) Neoplastic cells in the skeletal muscle (case 4) showing the pleomorphic neoplastic cells, usually with an eccentric reniform or indented nucleus (arrows) or with 3–4 multi-lobed nuclei or structures that resembled megakaryocytes (arrowheads)

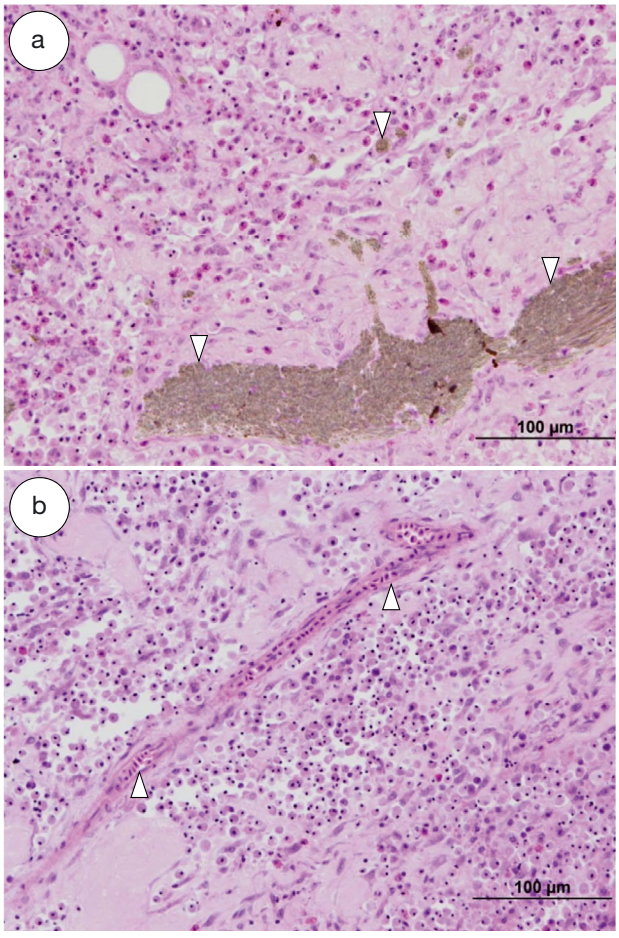

Fig. 6. Histological sections (H&E stain) showing host response and pathological observations in a putative histiocytic sarcoma in redfin needlefish. (a) Eosinophilic granulocyte infiltrations in the dermis (case 3); pigment cells (arrowheads) are found as a layer and aggregated within the inflammatory reaction. (b) Vascularization (arrowheads) in the skeletal muscle (case 4)

(Fig. 12a,c,e). Degenerative tumor cells had nuclei with dense chromatin, with some having a doughnut-like structure presumptively preceding karyolysis (Fig. 12c). Usually, the outer nuclear membrane had pores.

Organelles, such as abundant rough endoplasmic reticulum and mitochondria, were observed and Golgi apparatus and lysosomes were also seen. Cytoplasmic vacuoles were presumably formed from the coalesced structural remnants of either necrotic mitochondria or lysosomes that were found adjacent to the nuclei of degenerating or dying neoplastic cells. No obvious Birbeck granules were found. No obvious virus particles were observed.

4. DISCUSSION

Gross pathology, histopathological examination, IHC evidence, and ultrastructural features support the diagnosis that the nodular lesions in redfin needlefish are neoplastic, and best classified as a hematopoietic neoplasm, with characteristics of a putative HS. While the sampling methodologies used here allowed for detection and confirmation of this neoplastic lesion histologically at low prevalence, unavoidable delays in fixation might have led to post-mortem artefacts in histologic sectioned tissues. To mitigate this problem, histological evaluations were restricted to those specimens that were fixed within 24 h or less after capture. Following these protocols, unaffected tissue areas were found to be histologically normal even when

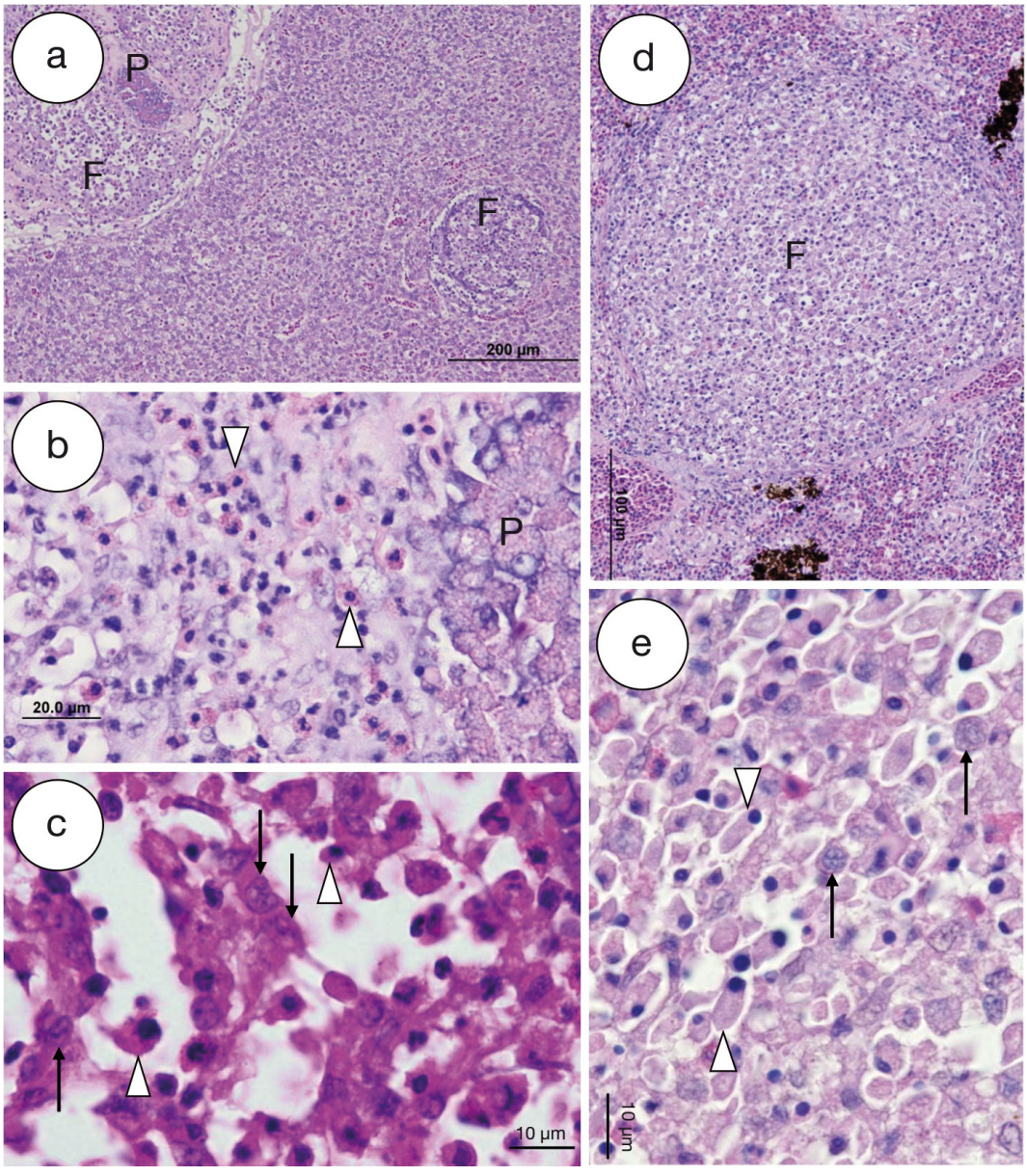

Fig. 7. Histological sections (H&E stain) of a putative histiocytic sarcoma possibly metastasized to the internal organs in redfin needlefish: (a) foci (F) in the liver parenchyma with view of exocrine pancreas (P; case 14); (b) moderate magnification of (a), showing mostly neoplastic cells, scattered eosinophilic granulocytes (arrowheads), and the exocrine pancreas (P); (c) higher magnification of (a), showing the focus of neoplastic cells in vital (arrows) or necrotic (arrowheads) condition; (d) focus (F) of neoplastic cells in the spleen (case 9); (e) higher magnification of (d), with neoplastic cells in vital (arrows) or necrotic (arrowheads) condition

the affected tissue area including the neoplastic lesions showed necrosis. Although only 1 fresh specimen was fixed within 6 h for routine diagnostics and TEM, valuable data were obtained in support of an HS diagnosis.

The neoplastic lesions documented here appear to be caused by the proliferation of atypical pleomorphic round-to-oval cells, with characteristics of histiocytes. In our study, it appears that following their origin, most likely in the skin tissue, the neoplastic cells do disseminate, spreading inwards to invade the skeletal muscle and then eventually possibly metastasizing to the spleen, liver, and posterior kidney. For example, the liver of 1 fish (case 13) had foci of alterations and some parenchymal vacuolations suggesting a preneoplastic condition. The cutaneous

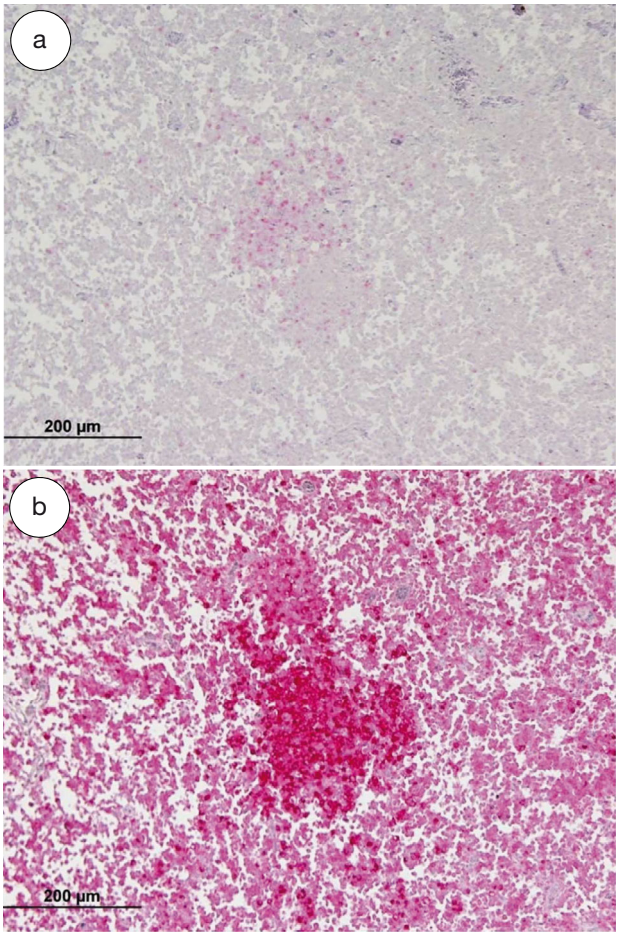

Fig. 8. Immunohistochemistry sections of a putative histiocytic sarcoma on the flank of a redfin needlefish (case 12): Ki67-positive (red) after (a) 0.5 h antibody incubation and (b) 24 h antibody incubation

tumor of northern pike is also derived from a histiomonocytic lineage that originates in the skin tissue, but metastasized tumor cells in internal organs (e.g. spleen, liver, and head kidney) were not detected (Thompson et al. 1987, Thompson & Kostiala 1990).

Some fish neoplasms, such as the hematopoietic neoplasms, are malignant (Schlumberger & Lucké 1948, Mawdesley-Thomas 1972). Metastatic cases have also been reported. In the flowerhorn cichlid (a hybrid of the Midas cichlid *Amphilopus citrinellus* and the threespot cichlid *Cichlasoma trimaculatum*), a cystic renal tubular adenocarcinoma had disseminated to the spleen (Rahmati-Holasoo et al. 2017), and a spontaneous lymphoma originating in the stomach was detected in the liver (Lin et al. 2008). Other cases include farm-raised Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss*, where an intestinal adenocarcinoma metastasized to the liver and spleen parenchyma, kidney interstitium, and cardiac lumen (Dale et al. 2009).

The presence of nuclear pleomorphism, active cell proliferation, and possible metastatic features suggest that the putative HS in redfin needlefish observed in this study can become malignant. However, this potential characteristic for metastases in other internal organs needs to be confirmed in a larger sample size of redfin needlefish cases exhibiting HS. Our putative diagnosis of the redfin needlefish hematopoietic neoplasm as HS is based on supportive histological, ultrastructural, and IHC evidence and comparative descriptions of similar tumors in other species, including humans. In part, this assessment is tentative because the nomenclatural system devised for hematopoietic neoplasia in human medicine (and other mammals) is a relatively complex and established classification scheme that does not easily apply to birds, reptiles, or fish, given the reduced morphological variability of this lesion type in these lower vertebrate groups (Jubb et al. 1993, Ferguson 2006).

We note here for clarification, that a caveat or weakness of this study is the broad specificity and limited sensitivity of the IHC tests. The antibodies used in the IHC assays were developed from mammalian systems (usually rodent models), and are readily available commercially, but there may be a potential for false positive readings, cross reactivity, or even absence of the equivalent antibodies in fish leading to a false interpretation of the results. Ideally,

Table 5. Immunohistochemical reactivity results of the putative histiocytic sarcomas (HS) occurring on the jaw and flank, and possibly metastasizing to the liver and spleen, shown by + (positive), – (negative), or nt (not tested) against each antibody

Case number	HS-affected tissue	Antibodies tested (hours of incubation)						
		Ki67 (0.5 h)	Ki67 (24 h)	Pan keratin (0.5 & 24 h)	NSE (0.5 & 24 h)	p53 (0.5 h)	S-100 (0.5 h)	CD163 (0.5 h)
11	Jaw	–	+	–	–	+	+	+
12	Flank	+	+	–	–	+	+	+
13	Jaw	nt	nt	nt	nt	+	+	+
14	Liver	nt	nt	nt	nt	+	+	+
9	Spleen	nt	nt	nt	nt	+	+	+

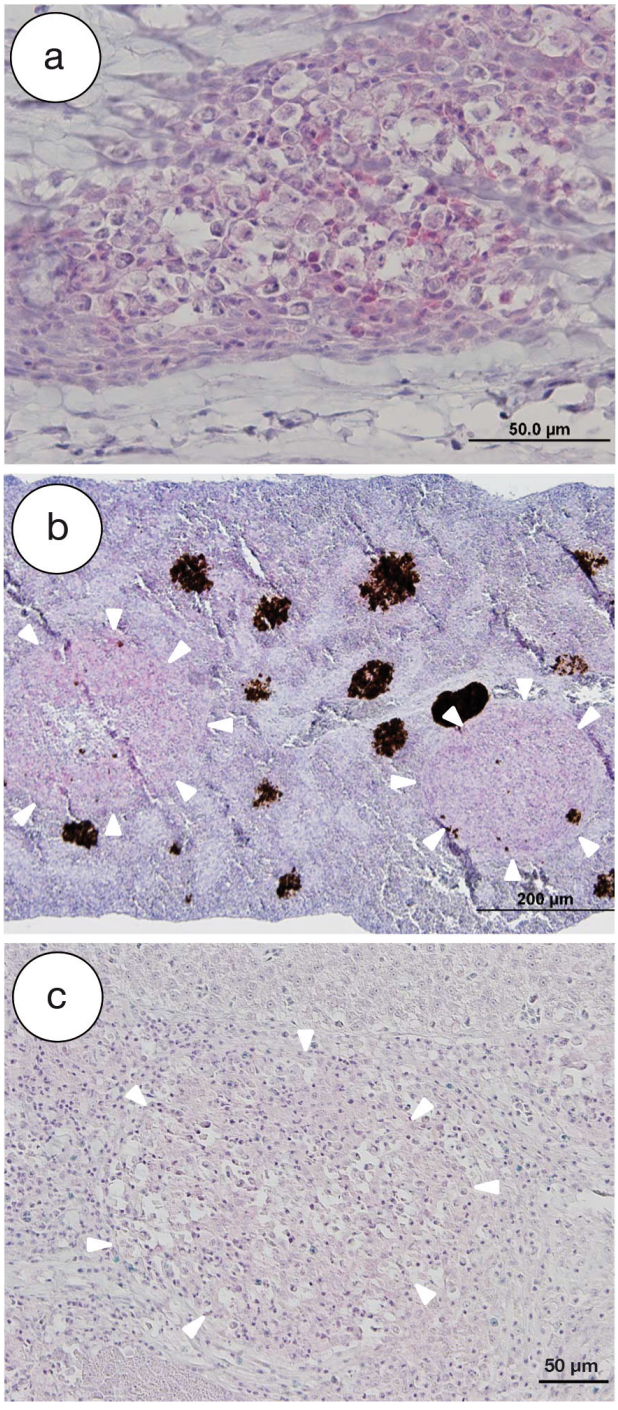

Fig. 9. Immunohistochemistry sections of redfin needlefish showing immunopositive p53 staining of neoplastic cells in (a) a putative histiocytic sarcoma in jaw tissue (case 13), and (b,c) possible metastases to the internal organs: (b) spleen with 2 foci (arrowheads) (case 9), and (c) liver with a focus (arrowheads) (case 14)

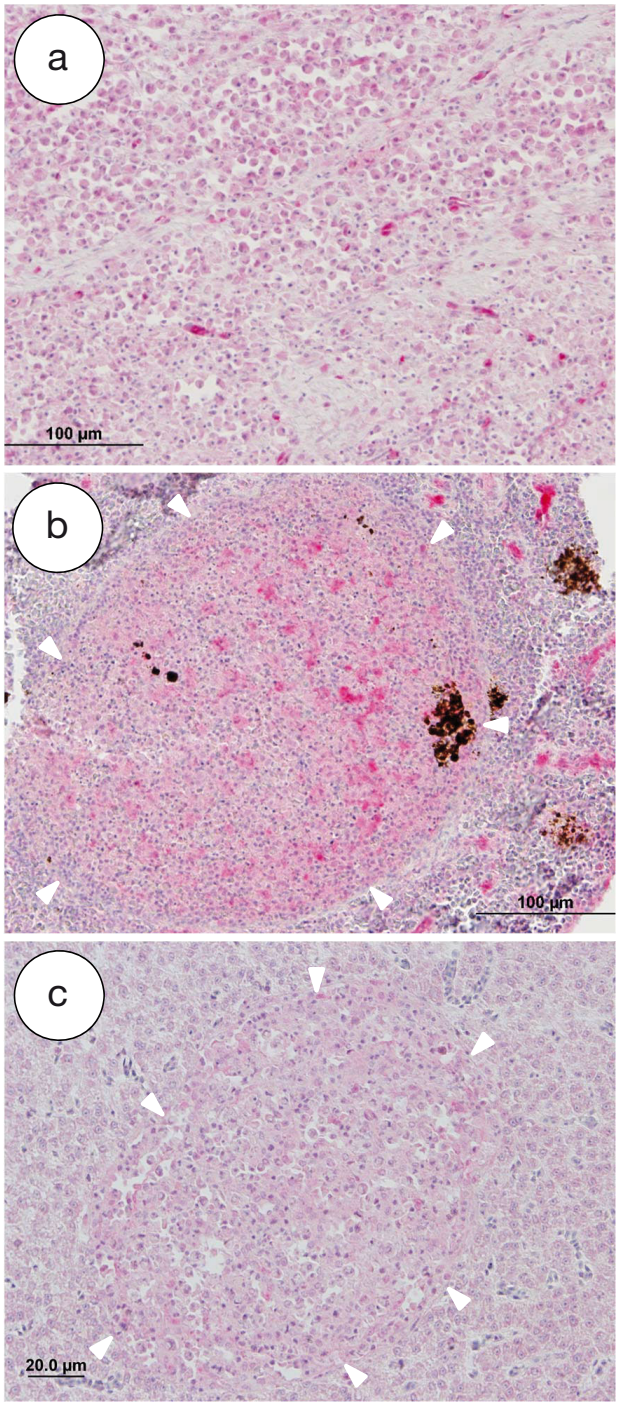

Fig. 10. Immunohistochemistry sections of redfin needlefish showing immunopositive S-100 staining of neoplastic cells in (a) a putative histiocytic sarcoma in jaw tissue (case 13; note that the strong reaction [bright red staining] of the arterioles could be cross-reactivity), (b) a possible metastasized spleen with a focus (arrowheads) (case 9; note that the strong reaction [bright red staining] of the blood vessels in the unaffected parenchyma could be cross-reactivity), and (c) a possible metastasized liver with a focus (arrowheads) (case 14)

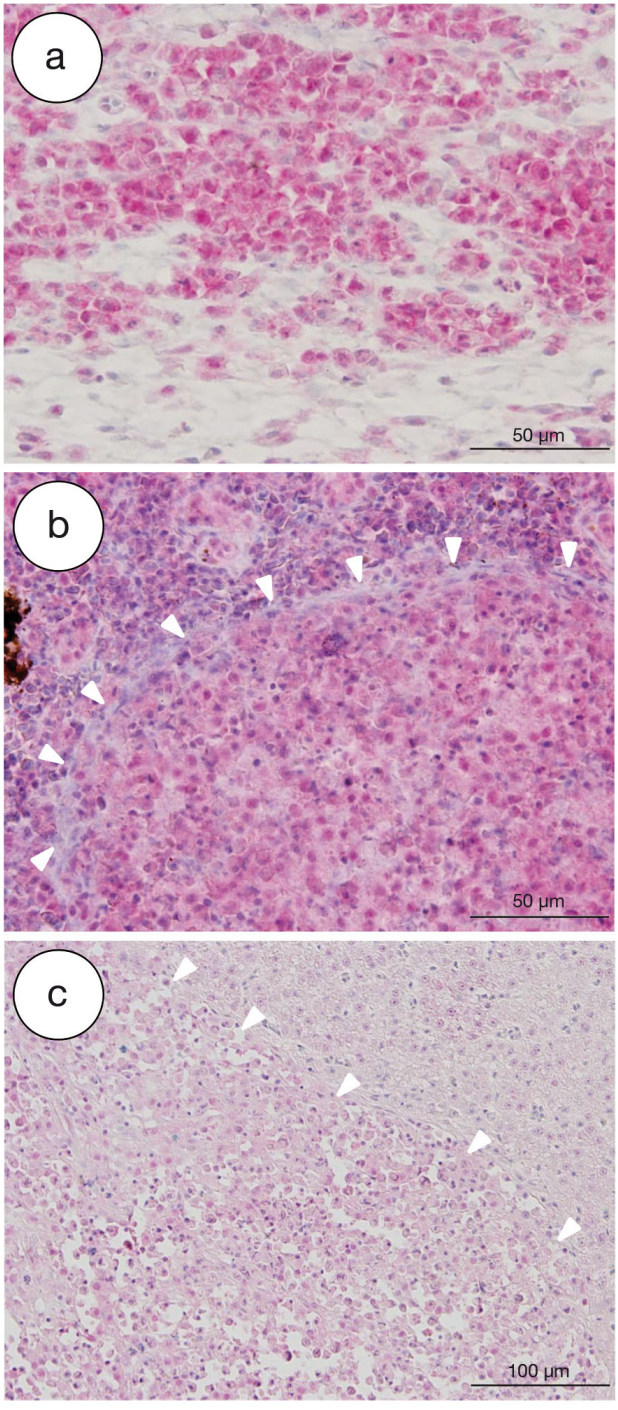

Fig. 11. Immunohistochemistry section of redfin needlefish showing immunopositive CD163 staining of neoplastic cells in (a) a putative histiocytic sarcoma in jaw tissue (case 13), (b) a possible metastasized spleen with a focus (arrowheads) (case 9), and (c) a possible metastasized liver with a focus (arrowheads) (case 14)

antibodies from marine fish should have been used for the IHC assays, but these were not easily available. However, it should be noted that many IHC studies (e.g. cell line characterization, cell lineage, tumor classification) of lower vertebrates use mammalian antibodies because of this reason, and they also use human positive control tissues because many of these biomarkers are conserved in vertebrates as proven by molecular studies (Nelson & Traub 1982, Herrmann et al. 1996, Tracy & Hedges 2000, Lane et al. 2010, Melo et al. 2015, Ueda et al. 2016). For example, NSE, Ki67, vimentin, desmin, fibronectin, cytokeratin, pancytokeratin, and proliferating cell nuclear antigen have been tested for and found to be positive immunocytochemically in fishes using commercially available mammalian antibodies (Zaccone et al. 1994, Parameswaran et al. 2007, Dale et al. 2009).

In human and veterinary medicine, the differential diagnosis of HS is contingent on verification of a histiocytic lineage with extensive immunophenotypical, cytological, and morphological diagnostics, distinction from other histiocytic processes, and through the exclusion of other, poorly differentiated, large cell malignancies (Pileri et al. 2002, Hornick et al. 2004, Vos et al. 2005, Takahashi & Nakamura 2013, Ansari et al. 2016). Morphologically, at the LM and TEM levels, the histiocytic nature of the neoplastic cells in the present study was also confirmed by the presence of reniform, infolded nuclei and by the absence of Birkbeck granules (characteristic of Langerhans cell histiocytosis/sarcoma; Deng et al. 2008). Inclusion of tumors as HS requires (1) neoplastic cell positive reactivity to CD68, CD163, and lysozyme, (2) weak or focal reactivity to S-100, (3) variable reactivity to Ki67, (4) negative reactivity to CD21 and CD35 (follicular dendritic cell sarcoma), and (5) lack of myeloid, B-cell, and T-cell markers (Pileri et al. 2002, Hornick et al. 2004, Vos et al. 2005, Dalia et al. 2014). A primary supportive IHC test for the diagnosis of HS in humans is the positive expression of CD163 (Vos et al. 2005, Takahashi & Nakamura 2013). CD163 is a hemoglobin scavenger receptor that is a specific marker for cells of monocytes and macrophage lineage (Lau et al. 2004). Our results confirmed CD163 positivity in 3 fish, which supports the diagnosis of HS for the tumor found in the redfin needlefish.

Another strong line of evidence for an HS diagnosis is that the neoplastic cells exhibited a positive reaction to S-100, indicating that these neoplasms must be derived from macrophages or are of Langerhans cell lineage. S-100 is also positive in HS (Deng et al. 2008). Further immunohistochemical and ultrastructural evidence from rarely reported cases of HS in northern pike also support our diagnosis (Thompson et al. 1987, Thompson & Miettinen 1988, Thompson & Kostiala 1990, Bogovski et al. 1994). Bogovski et al.

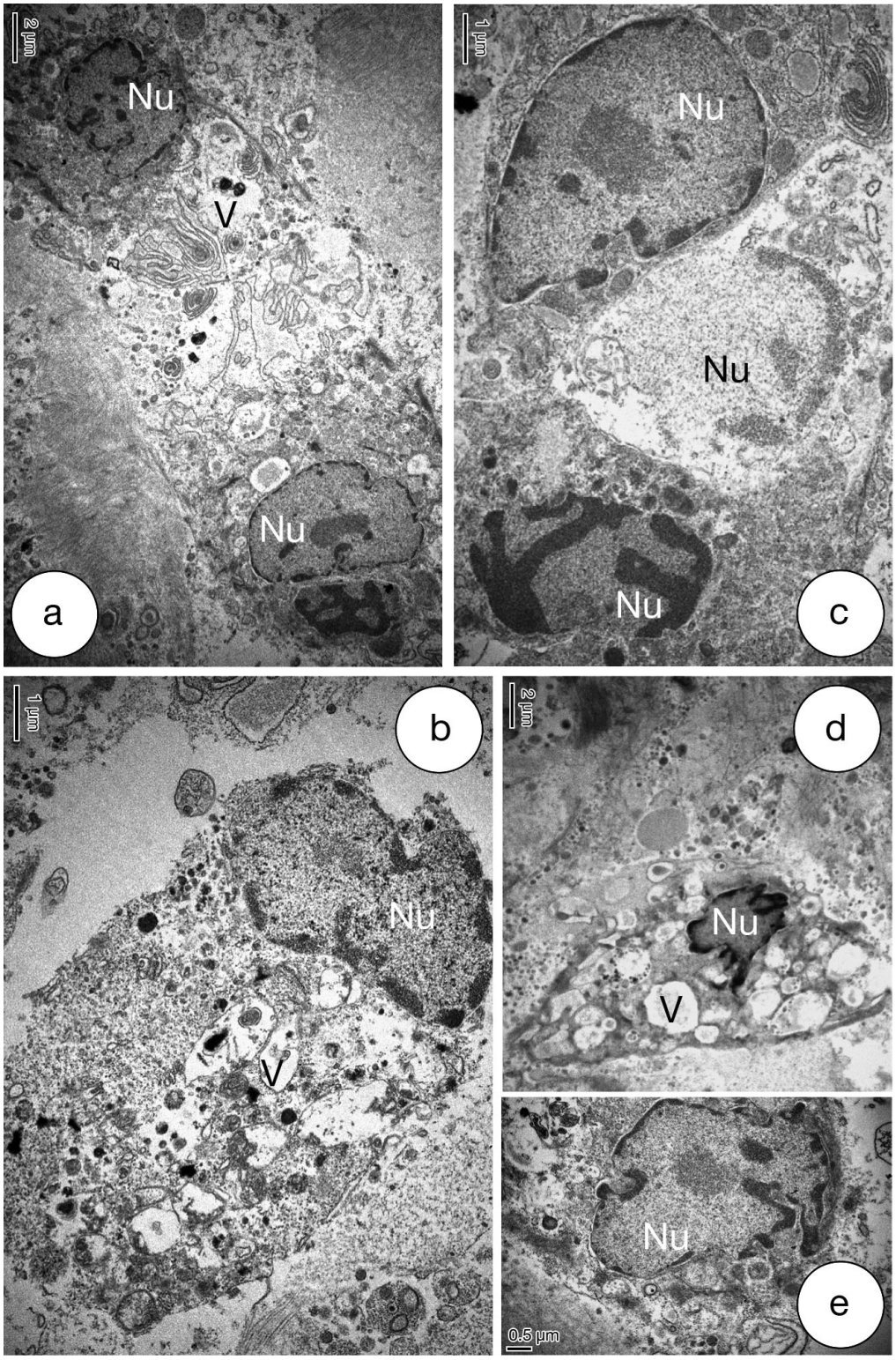

Fig. 12. Transmission electron micrographs of a putative histiocytic sarcoma in the jaw tissue of a redfin needlefish (case 13). (a) Overview of 2 proliferating neoplastic cells (nucleus [Nu]) with vacuolated (V) cytoplasm. (b) Typical neoplastic cell with an eccentric nucleus (Nu) and perinuclear cytoplasmic vacuolation (V). (c) Three stages of nuclear (Nu) degeneration of neoplastic cells. (d) Different view of a neoplastic cell with nucleus (Nu) and abundant vacuolar (V) cytoplasm. (e) Neoplastic cell showing the invaginated nuclear membrane

(1994) differentiated 3 histological types of neoplasm as subsets of lymphosarcoma (histiocytic lymphoma, histiocytoid-lymphocytic lymphoma, and lymphocytic lymphoma) occurring in northern pike by using IHC with rabbit anti-human polyclonal antibodies against lysozyme, alpha-1-antichymotrypsin, and S-100 protein. In their study, strong to weakly positive reactions to these antibodies were seen in the histiocytic lymphoma and histiocytoid-lymphocytic lymphoma, i.e. those types of lymphosarcoma that were associated with multinucleated giant cells and lymphocyte-like cells.

As expected, the Ki67 antibody test showed immunoreactivity in the neoplastic cells localized in both jaw and skin tissues after 24 h of incubation, indicating that the cells were proliferating, but the results were variable. Compared with the neoplastic cells located in the skin tissue, the stain was not taken up as rapidly by proliferating cells in the jaw tissue of one specimen (no reaction at 0.5 h of incubation), and the response after 24 h was light compared to the other specimen. The discussion here is based on only 2 specimens, and more confirmation is necessary, but 1 possibility for this difference is that the jaw tissues were slightly degraded. The neoplastic cells could have degenerated faster in the jaw tissue due to any of a few possibilities: (1) rapid postmortem changes, (2) differences between individual fish, and (3) because neoplastic cells in different tissue types (jawbone versus skin tissue) degenerate differently even in specimens preserved within 24 h of capture, as the 2 jaw specimens had been.

IHC expression of the tumor suppressor protein p53 is considered as a surrogate for mutation (Steele & Lane 2005). Thus, p53 IHC expression is generally used in the detection of tumors in human medicine (Franken et al. 2013, Ando et al. 2015), and has been demonstrated in e.g. fibrous histiocytomas, other mesenchymal tumors, and lymphomas (Said et al. 1992, Soini et al. 1992). The p53 gene is highly conserved amongst the animal kingdom, being found for example, in teleosts and invertebrates (Schmale & Bamberger 1997, Bhaskaran et al. 1999, McGladdery et al. 2001). Although the amino acid sequence of the p53 gene is characterized in various teleost species (Krause et al. 1997, Cachot et al. 1998, Luft et al. 1998, Brzuzan et al. 2009, Liu et al. 2011, Mai et al. 2012, Qi et al. 2013, Cheng et al. 2016), the function of p53 in teleosts is still not well understood. Mixed results for the p53 gene in teleosts and chondrichthyans have been obtained. For example, p53 was not detected in adult blue shark *Prionace glauca* tissues exhibiting hyperplasia, dysplasia, or neoplasia (Borucinska et al. 2008) nor in flowerhorn cichlids with cystic renal adenocarcinoma (Rahmati-Holasoo et al. 2017). Conversely, p53 mutations in teleosts have been reported in early-stage hepatic tumors (Cachot et al. 2000) and in contaminant exposures testing cellular proliferation responses (Blas-Machado et al. 2000, Yuan et al. 2017). Here, we also demonstrated a positive reaction to p53 antibody in the putative HS. Further study of the association of p53 with neoplasia in teleosts is necessary.

The IHC assays also supported the diagnostic findings that the neoplastic cells were of mesenchymal origin. Keratins and vimentin are intermediate filament proteins that are components of the cell cytoskeleton and are produced by epithelial and mesenchymal cell types, respectively (Hendrix et al. 1992, Fuchs & Weber 1994). A negative reaction to the pan keratin (for epithelial cells) antibody tests suggests that the neoplastic cell origin was mesenchymal (nonepithelial). Previous studies on fish tumor characterization have shown that epithelial tumors are conserved among vertebrates and the pan keratin test can be used in differential diagnosis. For example, Bunton & Wolfe (1996) used IHC to demonstrate the immune-histogenetic origin of various neoplasia induced by exposing medaka to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Mesenchymal neoplasms, rhabdomyosarcoma, teratoma, hemangiopericytoma, and fascial and undifferentiated sarcomas were negative to pan keratin antibody (AE1/AE3), while the epithelial neoplasm, a lepidocytoma (affecting scales), was strongly positive. Conversely, esocid lymphomas of mesenchymal origin were positive for vimentin and negative for keratin (Thompson & Kostiala 1986).

Diagnosis for the neoplastic lesions (HS) observed in this teleost species should be differentiated from the closely related lymphosarcoma that can superficially appear similar in histopathological evaluation. Lymphosarcoma occurs epizootically or spontaneously in wild and cultured fishes (primarily in esocids; Mulcahy 1970, Sonstegard 1975, 1976, Thompson 1982). Lymphosarcoma has been characterized grossly by single or multiple localized nodules, and histopathologically by a mass of proliferating, atypical lymphocytes in the skin tissue. The nodules arise from tissues of the head, trunk, fins, and jaw, but as the lesion progresses, the neoplastic cells spread deeper into the surrounding fibers of the skeletal muscle tissue, and eventually metastases can occur in organs, such as the spleen, liver, and kidney (Mulcahy 1963, 1970, Ljungberg & Lange 1968, Sonstegard 1975, 1976, Thompson 1982). Teleost and elasmobranch lymphosarcomas have been reported with multiple

descriptors: skin sarcoma, round-cell sarcoma, lymphoma, lymphoblastic lymphoma, epitheliotropic lymphoblastic lymphoma, or lymphoproliferative disorder (Ljungberg & Lange 1968, Bowser et al. 1985, Okihiro & Hinton 1989, Harada et al. 1990, Kieser et al. 1991, Manire et al. 2013).

Although the gross anatomical characteristics of the neoplasia and the invasiveness found in this study resembled epizootic lymphosarcoma in esocids and other teleost species, the histological, IHC, and ultrastructural morphological characteristics of the proliferating cells do not fit those of lymphocytes. The majority of proliferating cells appeared to be mononuclear, but cells with a lobulated nucleus or bizarre, megakaryocyte-like cells were also observed. Furthermore, TEM observations revealed that the nucleus of the neoplastic cell is indented or grooved, a primary characteristic of histiocytes, including those from teleosts (Pileri et al. 2002, Hornick et al. 2004, Vos et al. 2005, Deng et al. 2008), and different from the nuclear structure of lymphocytes in clinically healthy individuals.

Reactive inflammatory responses of EGCs as exudates were also observed in histological preparations of the neoplastic lesions described here. Tumors including HS in human cases and domestic animals may be accompanied by inflammatory cellular infiltrates (e.g. eosinophils, lymphocytes, neutrophils, and plasma cells) (Pileri et al. 2002, Hornick et al. 2004, Vos et al. 2005, Lau et al. 2008, Hao et al. 2010, Takahashi & Nakamura 2013, Moore 2014). In fish, reactive inflammatory lesions with EGCs have been associated with other tumor types, such as in neurofibroma in bicolor damselfish *Stegastes paritus* (Schmale et al. 2004).

The observation that the putative HS in redfin needlefish was least prevalent in the summer may be related to its life history. Although spawning is protracted throughout the year, it reportedly peaks in spring and summer, and juveniles are abundant during the same period (Breder 1932, Powell et al. 2007). This species apparently reaches maturity in its second year (age-1, at about 300 mm SL), with immature fish (age-0, at around 200 mm SL) far more common than older fish (Breder 1932). In our study, fish with neoplasms were primarily within the size range corresponding to the age-1 cohort of mature fish, with only 2 fish exhibiting neoplastic lesions whose SL fell into the age-0 mode and that could have been age-1 fish spawned late in the season. The length–frequency distribution of redfin needlefish in TB and CH, which consistently showed higher catch rates for age-1 than for age-0 fish, indicates that some biological or environmental factor(s) may contribute to the age class difference, or simply that areas inhabited by age-0 fish were not sampled. Sonstegard (1976) reported, for example, that lymphosarcoma in muskellunge reached the greatest prevalence from fall through spring, the breeding season for this species (Froese & Pauly 2014), and in most cases tumor growth progressed until fish death. As reported here, although the type of neoplasm and host species is different, Sonstegard (1976) also noted a low tumor prevalence in midsummer. Although not assessed here, this low prevalence was associated with a complete die-off of fish affected with lymphosarcoma in late spring (May and June) (Sonstegard, 1976). Seasonal trends in the prevalence of neoplasia can be the result of multifactorial interactions including (1) biotic factors such as spawning period, hormonal changes associated with metamorphosis (e.g. smoltification in salmonids), compromised immune systems, sensitivity to oncogenic viruses (Papas et al. 1976, 1977), or possibly mortality, and (2) abiotic environmental factors such as temperature (Anders & Yoshimizu 1994).

Nearly 50% of cutaneously initiated skin tumor cases in fish involve oncogenic viruses (Anders & Yoshimizu 1994, Ferguson 2006), and some hematopoietic neoplasms in fish are suspected to be of viral origin (Papas et al. 1976, 1977, Kent & Dawe 1990, 1993, Eaton & Kent 1992, Eaton et al. 1993, Miyazaki et al. 2000, Coffee et al. 2013). Although we were unable to attempt virus isolation in cell culture or neoplastic cell transplantation into healthy hosts, LM and TEM did not yield evidence of viral inclusion bodies or viral particles, respectively. However, we still cannot rule out a viral etiology for the putative HS in redfin needlefish and recommend a larger sample size of fresh neoplasms for ultrastructural and viral study. Virally-induced skin tumors are generally influenced by seasonal temperature fluctuations (Getchell et al. 1998), which could explain our observation of seasonal trends. In the case of esocid lymphosarcoma, a viral etiology was proposed because C-type particles were present in cell-free neoplastic homogenates and Koch's postulates were fulfilled in experimental transmissions (Mulcahy & O'Leary 1970, Papas et al. 1976, Gross 1983).

The nonrandom spatial trends in the prevalence of the putative HS in Florida populations of redfin needlefish are dramatically apparent (Fig. 1), suggesting that some location-specific factors or cofactors play an important role in the etiology of the lesion. That no cases of the putative HS were observed from redfin needlefish collected from SJR, CK, and AB is not surprising given the relatively small catches from

these cooler estuaries and the low overall prevalence of the lesions. The estuaries that constituted our study areas fit into 2 temperature categories that correspond to important differences in the abundance of redfin needlefish. As defined by the US Department of Agriculture (plant hardiness zones [PHZ] 9a–10a) from 1976 through 2005 (www.planthardiness.ars.usda.gov), the climate regions covering TB, CH, and northern IRL averaged annual extreme minimum temperatures of no less than –6.7°C, whereas in JX, CK, and AB (PHZ 8b and 8a) the average annual low temperatures ranged from –6.7°C to as low as –12.2°C.

The environmental factors and potential co-occurring etiological drivers that may influence the prevalence trends and distribution of the putative HS in redfin needlefish are not definitively inferable from our data. The tight clustering of catch localities of needlefish with the tumor in Riviera Bay (TB) and the mouth of the St. Sebastian River (southern IRL) is striking, but the much greater number of cases in the Banana River and their consistent occurrence through the study period suggest a role for unidentified tumorigenic factors in a possible multifactorial etiology (e.g. oncogenic viruses, chemical carcinogens, biotoxins) (Mix 1986, Malins et al. 1988). Perhaps not coincidentally, environmental stressors and possible carcinogenic cofactors such as poor water quality, harmful algal blooms, toxins, wastewater, nutrients, and microbial inputs, and chemical contaminants are an ongoing concern in these 2 systems, particularly the IRL (Trocine & Trefry 1996, Sigua & Tweedale 2003, Landsberg et al. 2006, Trefry & Trocine 2011, Gobler et al. 2013, Lapointe et al. 2015, Phlips et al. 2015).

Monitoring the status of animal health in an aquatic ecosystem is one means of assessing the overall health of the ecosystem. A high incidence of neoplasia in multiple species in the same ecosystem may be cause for concern. As well as putative HS in redfin needlefish in the IRL, this lagoonal system also has increased prevalences of gonadal tumors in hard clams *Mercenaria* spp. (Hesselman et al. 1988, Bert et al. 1993), fibropapillomas in green turtles *Chelonia mydas* (Lackovich et al. 1999, Foley et al. 2005, Hirama & Ehrhart 2007), and orogenital papillomas in bottlenose dolphins *Tursiops truncatus* (Bossart et al. 2005, 2017). Although unreported, a case of lymphosarcoma in a hardhead catfish, *Ariopsis felis,* captured from the IRL has also been observed (J. Fournie pers. comm.). It would be useful to conduct a comparative survey in the IRL for possible cofactors that may contribute to the etiology of these tumors in different species. Hypothetically, if linked, any environmental tumorigenic factors are of concern relative to biological and human health, and the IRL may prove to be a good model system for tumor research.

We have observed no redfin needlefish exhibiting the putative HS from any estuary since 2009 up until 2015. The IRL, the most likely location in which the putative HS would occur, has experienced dramatic declines in water quality during this time that may have contributed to a reduction in the redfin needlefish population. Likely triggered by aberrantly cold water temperatures and low rainfall in the winter of 2009/2010 (Phlips et al. 2015), these changes have included dramatic drops in the redfin needlefish population of the northern IRL, where the putative HS was observed most often in this study. Our ensuing low catch rate in this obviously important area prompted our decision to cut off our analyses in 2009, although FIM has continued to sample fish in all 6 estuaries. Thus, 2010–2016 data were censored out for computation of overall tumor prevalence (Table 3). Phytoplankton blooms (the so-called algal super blooms and brown tides observed in the IRL during 2011–2013) have co-occurred with, been part of, and perhaps directly driven some of the biological community shifts in the IRL, i.e. extensive losses in benthic submerged aquatic vegetation, primary producer communities, and low planktonic grazer community structure (Phlips et al. 2015). Hydrology and the biological processes it influences are perhaps the most important factors that set the IRL apart from TB and CH (Windsor & Steward 1987, Rao 1987, Phlips et al. 2015). The Gulf coast estuaries are relatively well mixed (McPherson et al. 1996, Morrison et al. 2011). In contrast, the IRL, and particularly the Banana River, is prone to stagnation and long pollutant residence times (Windsor & Steward 1987, Phlips et al. 2015).

Our results suggest that continued study of this lesion is warranted, and its morphological description should provide the basis for additional research. One useful aspect of robust, multi-estuary, multi-year fish population monitoring is illustrated by our results: that long-term FIM data sets can yield dividends beyond traditional fisheries data. Future efforts will focus on the IRL as a model system for tumor research with continued health monitoring of its fish populations. The epizootic is still ongoing, with a few redfin needlefish exhibiting external nodular growths still being reported from the IRL. A statewide epizootiological review of neoplastic lesions in aquatic animals is a logical next step, and geospatial trends in lesion prevalence will likely help reveal potential etiologic factors and research problems of the greatest importance for addressing environmental and human health concerns.

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