Gonadal Maturation and Maturity Staging of the Pacific Sand Lance *Ammodytes personatus* (Ammodytidae)

K. A. Zhukova[a, b, *] and M. R. Baker[c, d]

[a] Faculty of Biology, Shenzhen MSU-BIT University, Shenzhen, China
[b] Lomonosov Moscow State University, Moscow, Russian Federation
[c] School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA
[d] Friday Harbor Laboratories, University of Washington, San Juan Country, USA
*e-mail: kzh@fish-zoology.ru

Abstract—Detailed attributes on oogenesis, spermatogenesis, ovarian, and testicular maturity phases of the Pacific sand lance *Ammodytes personatus* are described here for the first time. Histology was subsequently used to define detailed morphological criteria related to five distinct histological and macroscopic maturity phases. This study revealed that Pacific sand lance do not exhibit sexual dimorphism in length and weight, however, statistically significant differences were noted for immature and mature fish. Maturation in Pacific sand lance occurred in females at a minimum *FL* of 97 mm and weight of 3.0 g. Maturation in males occurred at a minimum *FL* of 92 mm and weight of 2.5 g. First spawning was observed in females > 107 mm and > 3.5 g and in males > 104 mm and > 3.8 g. Bimodal size distributions were observed in mature ovaries, providing strong evidence for group-synchrony ovary organization, determinate fecundity, and the ability to estimate total fecundity prior to the onset of spawning. These results provide a useful reference for size-at-maturity and present a rigorous methodological approach to rapid and accurate assessment of reproductive maturation stages in this species. Moreover, the high-resolution maturity data provided in these analyses may be applied to inform stock-recruitment relationships and to estimate effects of parental stock composition on reproductive potential. The histological description of gonadal morphology may species be relevant to the reproductive biology of other members of family *Ammodytidae*, a closely related group of fishes.

Keywords: the pacific sand lance *Ammodytes personatus*, reproduction, gonadal maturity phases, ovaries, testes, histology

The pacific sand lance *Ammodytes personatus* (Fig. 1) is a marine fish species of the family Ammodytidae, widely distributed in the eastern North Pacific Ocean, California Current, Gulf of Alaska, Aleutian Islands, and the eastern Bering Sea (Orr et al., 2015). As an important forage species in North Pacific food webs (Whitehouse et al., 2014, 2021), it occurs in the stomachs of top predators, ranging from ground and pelagic fishes to marine birds and mammals (Wilson et al., 1999; Chereshnev et al., 2001). Pacific sand lance is a planktivore, consuming predominantly calanoid copepods and other mesozooplankton (Tokranov, 2007; Sisson and Baker, 2017). This species employs a strategy of energy conservation, exhibited on diel and seasonal (Baker et al., 2019) cycles, including resting periods at night and a winter dormancy stage, where the fish remain buried in bottom sediments (Ciannelli, 1997; van Deurs et al., 2010). These fish overwinter in sediments and use energy reserves acquired the previous spring and summer (van Pelt et al., 1997) for maturation of the gonads and maintenance of metabolism

(Winslade, 1974). Similar to other species of *Ammodytidae* (Macer, 1966; Scott, 1972), spawning takes place once per year during the months of November–March (Robards et al., 1999a), intertidally on fine gravel or sandy beaches where individuals spawn demersal eggs (Pinto, 1984; Pierce et al., 2012). The pelagic larval stage lasts approximately one month, and young sand lance (≥25 mm standard length) settle in nearshore benthic habitats (Doyle et al., 2009; Matarese et al., 2003).

Although many aspects of pacific sand lance reproductive biology have been elucidated, there is no comprehensive analysis or description on gonad development necessary to determinate reproductive status, staging, and potential. Furthermore, information on the gonadal development gonads of representatives of this family of fishes (*Ammodytidae* spp.) is scarce (Robards et al., 1999a, 1999b; Velikanov and Stominok, 2001). Any histological studies of germ cells are absent, with the exception of Okamoto et al. (1989), who gives some illustrations of *Ammodytes japonicus*
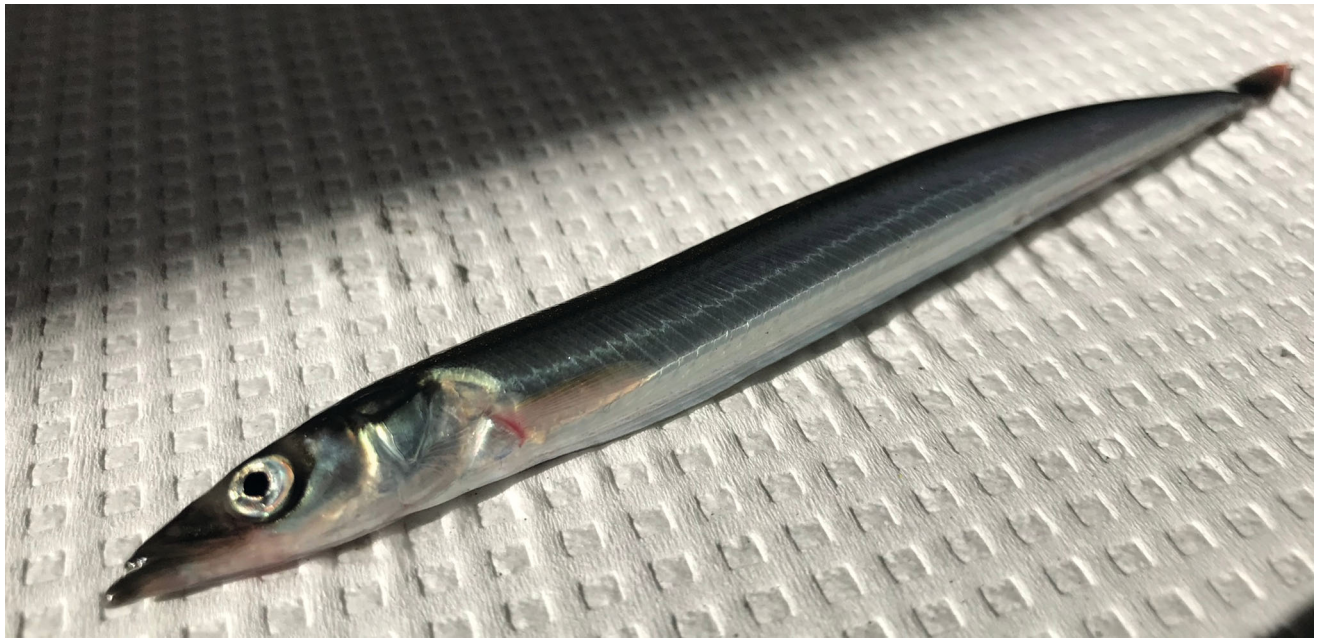
Fig. 1. Pacific sand lance *Ammodytes personatus*, photo M.R. Baker.

(according Orr et al., 2015, systematic) ovary histology. Knowledge on gonadal maturation is required to establish the onset and duration of spawning season, the size and age at maturity, and spawning pattern.

The aim of the study was to undertake histological investigation of the development stages of growing female and male germ cells, create macroscopic scales, and elaborate identification criteria of each maturity phase. The maturity classification presented here can be used by trained observers onboard research and commercial vessels to identity phase of pacific sand lance gonadal maturity accurately. The maturity scale also will help to separate investigated species between mature and immature, which is necessary for establishing maturity ogives traditionally applied in analytical stock assessments to compute spawning stock size.

MATERIALS AND METHODS

All fish analyzed were collected from October to December 2017, and in March 2018 in the San Juan Archipelago in the Salish Sea, USA. Fish were collected primarily from an offshore dynamic sand wave field bedform in the San Juan Channel (48°31′ N, 122°57′ W, $n = 71$). This is a benthic habitat approximately 1.75 km long and 0.4 km wide, known to consistently support large populations of pacific sand lance (Greene et al., 2020, 2021; Baker et al., 2021). Fish at this site were collected directly from sand sediments, using a van Veen grab sampling device. Fish were also collected with seine nets from two nearby by beach sites at Jackson Beach (48°31′ N, 123°00′ W, $n =$

23) and South Beach (48°27′ N, 122°59′ W, $n = 30$), located at a distance of 4.5 km and 6.5 km from the offshore site, respectively. After fork length (*FL*) and body weight (*W*) measurement whole fish samples were preserved in formalin for subsequent histological analysis.

Histology. Histological analysis was performed on 124 gonads of different maturity phases. Gonads were harvested, photographed and fixed in 10% formalin solution in the laboratory. The samples were dehydrated, cleared with xylol, embedded in paraffin, sectioned at 3−5 μm, and stained with hematoxylin and Ehrlich's eosin. The OLYMPUS BX45 microscope and Leica DC 100 digital camera were used for microscopy and photography. Image Processing and Data Analysis in Java (ImageJ) was used for measuring the diameters of germ cells and visualizing their structures. The maturity of each gonad was classified, based on the most advanced oocytes stage observed in the histological sections according to scale described in Brown-Peterson et al. (2011). After histological examination of the gonad, 39 females and 21 males were identified as immature, 4 females and 5 males—early developing subphase; 10 females and 20 males—developing phase; 10 females and 9 males—spawning capable; 5 females and 1 male—regenerating.

Oocyte diameters and size−frequency distribution were determined in 5 immatures ($n = 623$ oocytes), 4 early developing ($n = 396$), 5 developing ($n = 303$), 5 spawning capable ($n = 198$) and 5 regenerating ovaries ($n = 332$). Diameters of all oocytes, in which nucleus was visible in the section (1852 oocytes), in

histological cross-sections contained in 3-mm^2 frame were measured.

The external appearance of the gonads at each stage are described here because visual staging scales are commonly used in surveys and field studies to estimate fish sexual maturity. The visual schematic presented was performed through histological determination of gonadal phases.

The nuclear-cytoplasmic ratio (N/C) was calculated as a ratio of the nucleus diameter to germ cell diameter. The Kruskal−Wallis one-way ANOVA was used to assess the significance of differences in the average values of *FL* and *W*. Visual scales for ovaries and testes maturity were performed according to histological analysis.

RESULTS

Pacific sand lance had paired gonads, which were longitudinally oriented under the intestine and joined caudally. The ovaries were suspended from dorsal body wall by the mesovarium; the testes were attached by mesorchium. Their shape and size strongly changed in the process of their development during the annual reproductive cycle (Fig. 2). The ovaries of pacific sand lance were of the cystovarian type, and had an ovarian cavity and oviduct. The testes were unrestricted spermatogonial type according Uribe et al. (2014), and characterized by the occurrence of spermatogonia along the lengths of the testis lobules.

The phases of maturity of each ovary and testis were determined from histological section and whole oocyte and germ male cell characteristics. Histological analysis allowed distinguishing five phase of gonadal maturity: immature, early developing subphase, developing, spawning capable and regenerating, which differed in composition and condition of germ cells.

Females. Immature ovaries were small and elongated with rounded edges and located at the caudal section of the female body (Fig. 2). Histologically, they contained only oogonia and perinuclear oocytes; both stage of germ cells occurred in all investigated ovaries. Oogonia had an ovoid shape, a hyaline ooplasm with a diameter of approximately 7−8 µm. The nucleus was spherical and large (N/C about 71%) with a diameter of 5−6 µm and contained a single nucleolus. Oogonia were scattered in the germinal epithelium as individual cells and presented in all investigated ovaries (Fig. 3). Perinucleolar oocytes were bigger with a mean diameter of 45.5 µm ± standard deviation (*SD*) 15.4. Perinucleolar oocytes had deeply basophilic ooplasm, spherical shape and a large nucleus in the central position with a diameter of 23.0 µm ± 7.8 *SD*, N/C 52.8% ± 10.3 *SD* (Figs. 3a−3c, 3e, 3f). Perinucleolar oocytes and oogonia showed a unimodal size−frequency distribution (Fig. 4).

Early developing subphase ovaries were small, but easily distinguishable, restricted to posterior body cav-
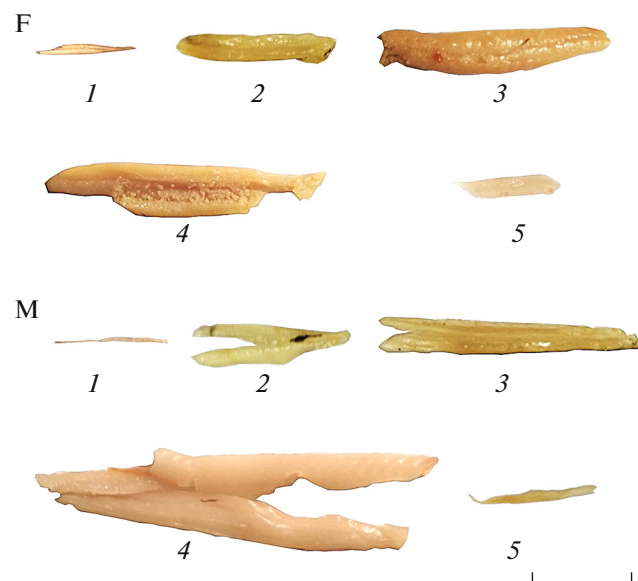

Fig. 2. Pacific Sand lance ovaries (F) and testes (M) of different gonadal phases: *1*—immature, *2*—early developing subphase, *3*—developing, *4*—spawning capable, *5*—regenerating. Scale: 1 cm.

ity. These were 3× larger than immature ovaries and occupied approximately a quarter of the body cavity. Such gonads contained cortical alveoli and primary vitellogenic oocytes. Cortical alveoli oocytes had a mean diameter of 150.4 µm ± 41.0 *SD*, light staining basophilic ooplasm. Cortical alveoli were spherical in shape and varied in size, with the largest reaching 12 µm; cortical alveoli were located in the periphery of the ooplasm, under the pellucida zone. Nuclei were round in shape with a slightly folded border; N/C was 32.8% ± 7.7 *SD* (Figs. 3b, 3c). The diameter of primary vitellogenic oocytes was 182 µm ± 26.6 *SD*. Ooplasm became less basophilic and were filled by cortical alveoli with a diameter of approximately 10−16 µm. Small irregular yolk granules occurred in the ooplasm periphery and had a diameter range of 2 to 3 µm. Zona pellucida thickness was 2 µm. Nuclei has a slightly folded border; N/C was 21.9% ± 5.5 *SD* (Fig. 3b). Oocyte size−frequency analysis revealed a bimodal distribution (Fig. 4).

In developing phase, ovaries were enlarged and extended to the mid-body cavity, occupying approximately 50−70% of the total body cavity volume. The advanced germ cells in developing gonads were secondary vitellogenic oocytes, with a mean diameter of 274.0 µm ± 41.7 *SD*, N/C was 17.9% ± 4.3 *SD*. Cortical alveoli were also enlarged, with a diameter increased to 20−25 µm. Small yolk globules (2−3 µm) presented in the periphery and bigger ones (6−8 µm) were observed in the middle area of the ooplasm. Zona radiata were 2−3 µm in thickness and had radial striations (Fig. 3c). Secondary vitellogenic oocytes showed a relatively simultaneous substantial increase in size
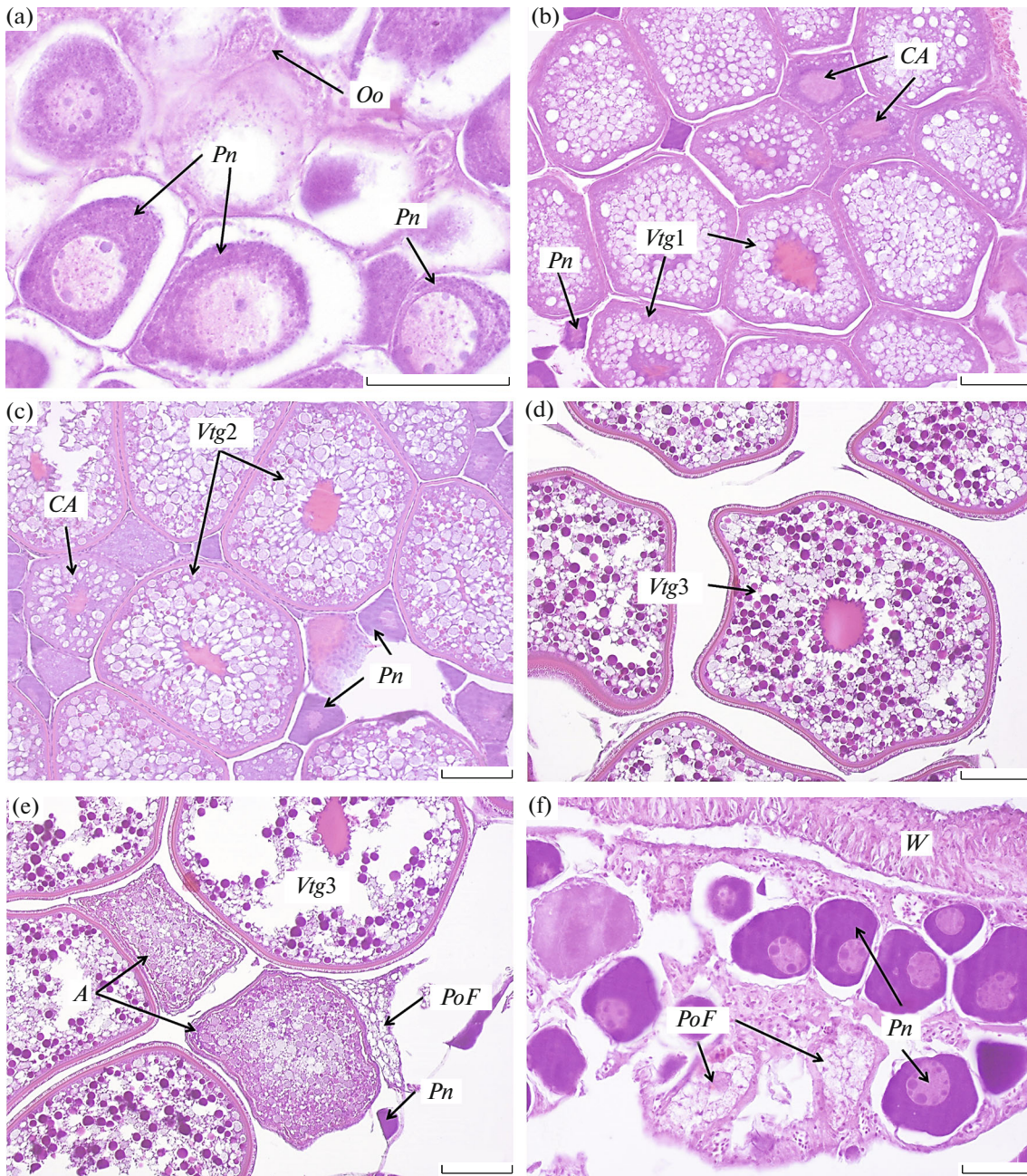
Fig. 3. Pacific Sand lance ovaries of different gonadal phases: (a) immature, (b) early developing subphase, (c) developing, (d, e) spawning capable, (f) regenerating. Microscopic characteristics include oogonia (*Oo*), perinucleolar (*Pn*), cortical alveoli (*CA*), primary (*Vtg*1), secondary (*Vtg*2), tertiary vitellogenic (*Vtg*3), postovulatory follicles (*PoF*), atretic eggs (*A*), ovarian wall (*W*). Scale: (a, f) 50, (b–e) 100 μm.

during vitellogenesis from 180−219 μm at the middle of developing phase to 270−300 μm at the end of that phase. Only minor asynchrony was visible in the development of the advanced cohort of oocyte (Fig. 4).

The ovaries of the spawning capable females reached their maximum size, extending into the anterior body cavity. The tertiary vitellogenic oocytes had a diameter of 359 μm ± 56.5 *SD* and N/C 15.6% ± 3.3 *SD*. The diameter of yolk globules ranged between 5 and 15 μm; these were distributed throughout the ooplasm. Zona radiata with distinct radial striations was thicker (5 μm) (Figs. 3d, 3e). Germ cell size-frequency analysis revealed a large gap between the perinucleolar oocyte reserves (<100 μm) and tertiary vitellogenic oocytes cohort (>300 μm; Fig. 4). Atretic eggs presented in some gonads. Where present, these were characterized by oocytes progressively shrinking and fragmentation in the zona pellucida. During atresia,
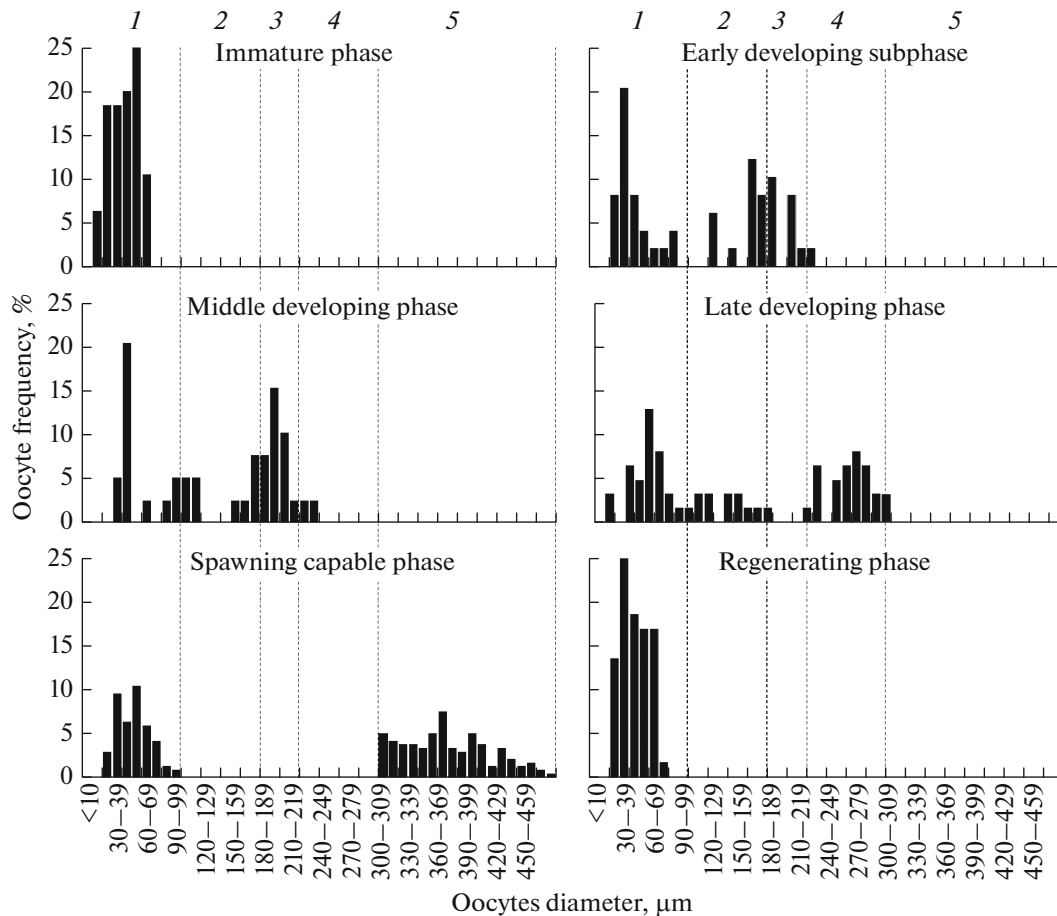
Fig. 4. Oocyte size–frequency histograms of ovarian development representative of each reproductive phase in female Pacific sand lance, including: *1*—primary growth, *2*—cortical alveolar, *3*—primary, *4*—secondary, *5*—tertiary vitellogenic oocytes (*n* = 1852).

follicular cells became hypertrophic and gradually and eventually engulfed the oocyte content during the various stages of resorption (Fig. 3e).

Gonads of the regenerating phase were flaccid, empty and bloodshot in appearance. Histologically, ovaries contained perinucleolar oocytes and postovulatory follicles, which remain in ovarian lamellae after ovulation and were formed by the follicular cell layer and the theca. Abundant blood vessels were present (Fig. 3f). Oocyte size-frequency became unimodal, similar to the immature phase (Fig. 4).

Males. In pacific sand lance immature testes were small, thin and elongated with acuminate edges. This phase was characterized by the presence only spermatogonia in testes (Fig. 5a). Primary spermatogonia were the largest germ cells with diameters of 7−8 μm. These cells were spherical in shape with light granular cytoplasm. The nucleus was in the central position, with a diameter ranging 4−5 μm and a single nuclei, approximately 1−2 μm in width. Late spermatogonia were smaller with a diameter range 5−6 μm; these exhibited similar morphological characteristics as pri-

mary spermatogonia and were enclosed within a cyst comprised of surrounding Sertoli cells. Spermatogonia were presented in all testes.

Early developing subphase male gonads were larger than the immature stage and easily distinguishable in the posterior part of body cavity. Histologically, testes contained spermatogonia, including primary and secondary spermatocytes (Fig. 5b). The diameter of primary spermatocytes was 4−5 μm. Secondary spermatocytes were spherical in shape with a spherical nucleus; the diameter was approximately 3 μm.

Testes of the developing phase were typically thicker and longer and occupied approximately half of the body cavity and were dense to the touch. Such gonads contained male germ cells of all phases of maturation from spermatogonia to spermatozoa, however spermatocytes and spermatids prevailed in number (Fig. 5c). The size of spermatids did not exceed 2 μm. Spermatozoa had oval-shaped heads, the length of which did not exceed 2 μm, and a width of 1 μm.

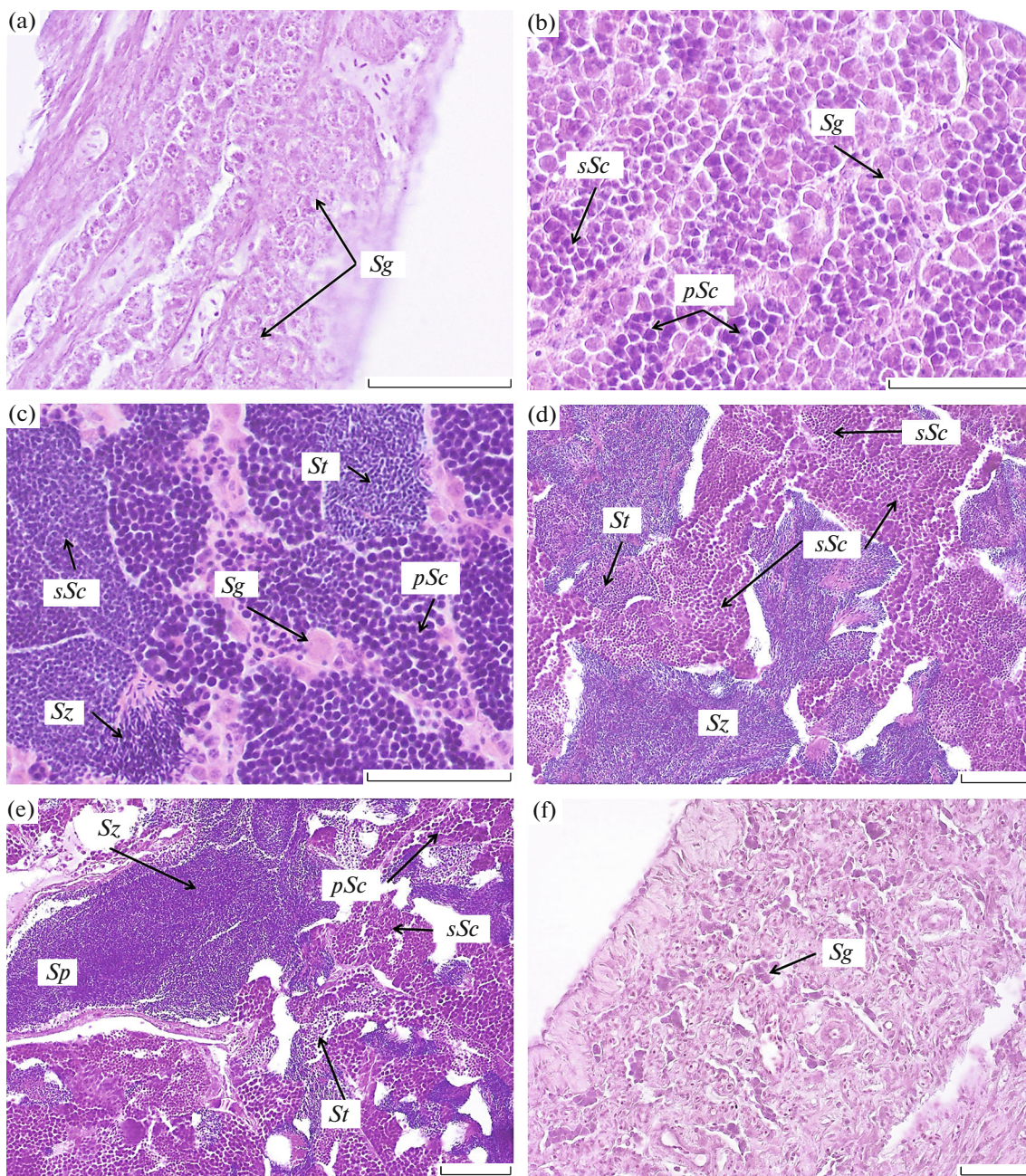Spawning capable testes reached their maximum size and occupied nearly the entire volume of the body

Fig. 5. Pacific sand lance testes at different gonadal phases, including: (a) immature, (b) early developing subphase, (c) developing, (d, e) spawning capable, (f) regenerating. Microscopic characteristics include spermatogonia (*Sg*), primary (*pSc*), secondary spermatocytes (*sSc*), spermatids (*St*), spermatozoa (*Sz*), spermatoduct (*Sp*). Scale: 50 μm.

cavity; active spermiogenesis was ongoing in these gonads. The massive pools of spermatozoa were released from the cysts due to spermation (Fig. 5d) and accumulated in the spermatoduct (Fig. 5e). The walls of the seminiferous tubules were strongly stretched, whereas some small cysts of spermatids and spermatocytes were still present (Figs. 5d, 5e).

Regenerating testes from postspawning males were flat and flaccid. Lobules of the testes were smaller and interstitial tissue had become noticeably thicker. Only spermatogonia proliferation was observed in cysts (Fig. 5f).

Based on reproductive differences, all studied individuals were divided into 2 groups: immature and mature. Immature gonads were observed in the smallest fish, which had a mean *FL* and *W* of 81.7 mm $\pm$ 12.1 *SD* (65.0−83.5 mm) and 1.7 g $\pm$ 0.6 *SD* (0.8−3.1 g) for female and 82.8 mm $\pm$ 11.0 *SD* (66.0−98.0 mm) and 1.8 g $\pm$ 0.7 *SD* (0.9−3.3 g) for males. *FL* and *W* of individuals with developing, spawning capable and

regenerating gonads were not statistically significant different, and were therefore evaluated and categorized as a comprehensive mature group. Mature females had a mean *FL* and *W* of 117.1 mm ± 10.0 *SD* (97.0−135.0 mm) and 5.5 g ± 1.5 *SD* (3.1−8.8 g). Mature males were similar with a mean *FL* and *W* of 111.2 mm ± 12.1 *SD* (92.0−153.0 mm) and 4.7 mm ± 1.8 *SD* (2.4−12.2 g). There was no statistically significant difference between two sexes in either *FL* or *W* within immature and mature groups. However, significant differences in *FL* and *W* were observed between immature and mature individuals ($p \leq 0.001$).

Maturation in pacific sand lance gonads occurred in females at a minimum FL of 97 mm and *W* of 3−4 g, and in males at a minimum *FL* of 92 mm and *W* of 2.5−3 g. First spawning was observed in females >107 mm and >3.5 g and in males *FL* > 104 mm and *W* > 3.8 g.

DISCUSSION

The histological characteristics used to determine ovarian development have allowed us to establish unique criteria for each reproductive stage of pacific sand lance (Table 1). We distinguish four phases of reproductive development—immature, developing, spawning capable, and regenerating—as well as one subphase—early developing. These reproductive stages may now be distinguished at the macroscopic scale of analysis of the gonads, without the need for microscopic examination. Further, several basic criteria associated with easily distinguished visual characteristics have also been identified, each correlated to a distinct reproductive stage. These criteria provide reliable predictive metrics for assessing reproductive maturity status, based on a coarse examination of these fish, including morphology (the length of specimen), gonad size and shape, volume occupied by the gonad in the body cavity, and oocyte visibility. These metrics will enable rapid assessment of maturity state in this species and might be used to further life history analysis and to inform applied management at broader scales.

The bimodal size distribution witnessed in mature ovaries indicates that pacific sand lance has group-synchronous ovary organization. The first cohort represented by previtellogenic oocytes, serves as the reserve fund of germ cells. The second cohort is represented by vitellogenic oocytes, which will be spawned during the current breeding season. Pacific sand lance has determinate fecundity. In such species total fecundity is defined prior to the onset of spawning. Similar reproductive characteristics have been observed in lesser sand−eels *Ammodytes marinus* in the North Atlantic Ocean (Boulcott and Wright, 2008). Oogonia and perinuclear oocytes presented in ovaries of all maturation phases in a large amount (38−51% of the total number of cells in ovary); this phenomenon is typical in iteroparous species (Murua and Saborido-Rey, 2003).

Developmental staging that occurred in the gonads of pacific sand lance are the similar to those described for other species with group−synchronous development. The volume of oocytes increases rapidly while the nuclear−cytoplasmic ratio decreases obviously with the maturation and growth of oocyte. This is because the volume of the cytoplasm increases faster than the nucleus. In immature ovaries, only previtellogenic oocyte growth is observed, during which the diameter of germ cells changes only slightly, in comparison to levels of variation during vitellogenesis. The beginning of yolk accumulation in oocytes, and, accordingly, the transition from an immature to mature state is marked in early developing subphase gonads, which is associated with a slender growth of the ovaries. Active vitellogenesis, which significantly increases oocyte diameters during developing phase, ends at spawning capable gonads. During active spawning, subphase oocytes increase in size more than in 2.5 times due to hydration, and ovulation.

Perinucleolar oocytes and primary vitellogenic of pacific sand lance analyzed here had similar morphology and diameters to those in sand lance sampled in Northern Hokkaido (likely *Ammodytes hepaterus*, though possibly *A. heian* or *A. japonicus*; measured Fig. 4: perinuclear oocytes 64.9 μm ± 19.3 *SD*, primary vitellogenic oocytes 182.9 μm ± 15.0 *SD*), shown Okamoto et al. (1989). Atresia was detected in some gonads; however, it was partial and affected no more than 20% of oocytes in ovaries. Such low incidence of atresia is natural level which regulated annual fecundity (Lubzens et al., 2010).

Immature testes were recognizable as having the smallest size and contained spermatogonia. The initiation of spermatogenesis occurred in the early developing subphase and marks the onset of maturation. The spermatogonia enters the first divisions of meiosis, resulting in two primary spermatocytes; the second meiosis division of each primary spermatocytes ends with the appearance of two second spermatocytes. Simultaneously, the size of testes increases ×2. Active spermatogenesis continues at this stage and spermiogenesis begins in testes in developing phase, which in pacific sand lance, is associated with an impressive growth of the testes. During active spermiation, the spermatozoa are released into the lobular lumen that is in continuity with that of the efferent ducts (Fig. 5e). In spawning capable gonads, there were abundant spermatozoa in the lobular lumen, however cysts with spermatogonia, spermatocytes and spermatids remained along the lobules. The presence of cysts with less advanced germ cells may enable males to engage in multiple spawning events in an extended spawning interval, lasting for a month, or possibly longer, as suggested in Robards et al. (1999a). After spawning, only spermatogonia remain in the regenerating gonads and the whole spermatogenesis begins once again. In this regard, testes development in pacific sand lance has many similarities with neote-

Table 1. Macroscopic maturity scale for gonads of the pacific sand lance *Ammodytes personatus*, with corresponding histological gonadal phases and oocytes stages

Maturity phases	Features	
	main histological	main macroscopic
Ovaries		
Immature	PG	Small, flaccid, translucent, distinguishable from testes by thicker appearance and rounded edges. *FL* < 96 mm
Early developing sub-phase	PG, CA, some Vtg1	3× larger than immature, occupy about 1/4 the volume of the body cavity, opaque, no eggs are visible to the eye
Developing	PG, CA, Vtg1, Vtg2	Opaque, orange, pink, occupy about 1/2–2/3 the volume of the body cavity. Small eggs are poorly visible to the eye, with diameter < 0.3 mm
Spawning capable	PG, CA, Vtg3	Turgid, voluminous, occupy the entire volume of the body cavity. Eggs are clearly visible to the eye, with diameter >0.3 mm
Regenerating	PG, PoF	3× times larger than immature, opaque, flaccid, occupy <1/4 the volume of the body cavity
Testes		
Immature	Sg	Small, translucent, flat, distinguishable from ovaries by the lanceolate shape and acuminate edges. *FL* < 91 mm
Early developing sub-phase	Sg, pSc, sSc	Opaque, less flaccid, shape lanceolate, occupy about 1/4 the volume of the body cavity
Developing	Sg, pSc, sSc, St, Sz	Opaque, lanceolate shape, dense to the touch, occupy about 1/2 the volume of the body cavity
Spawning capable	Sg, pSc, sSc, St, Sz prevail	Voluminous, occupy the entire volume of the body cavity, opaque and milky-white
Regenerating	Sg	Flat, flaccid, lanceolate, opaque, occupy <1/4 the volume of the body cavity

Histological features include primary growth (PG) oocytes; cortical alveolus stage (CA) oocytes; primary (Vtg1), secondary (Vtg2), and tertiary vitellogenic (Vtg3) oocytes; postovulatory follicles (PoFs); spermatogonia (Sg), primary (pSc), secondary spermatocytes (sSc), spermatids (St), spermatozoa (Sz); *FL*—fork length.

leosts with unrestricted spermatogonial testis type (Uribe et al., 2014; Grier et al., 2016).

Females and males were not found to differ in size. However, significant differences in length and body weight were observed between immature and mature cohorts, which is consistent with other studies in pacific sand lance (Robards et al., 1999a). In contrast, in *Ammodytes marinus*, mature females were documented to be heavier than males (Gauld and Hutcheon, 1990). It remains to be seen whether or not there are differences in the relative timing of maturation. While it appears both males and females are on approximately similar timeframes of development for winter spawning, there may be subtle differences in the relative timing of maturation between the sexes, an important detail to consider in assessing relative fecundity in individuals and overall spawning potential in stocks.

Our results confirm similar trends and in size-at-maturity as other species in this genus. The first sign of oocyte recruitment and secondary growth were observed in females in our study of *Ammodytes personatus* at approximately the same sizes—97–100 mm, as noted in *Ammodytes marinus* from the north-western North Sea (Gauld and Hutcheon, 1990) and in *Ammodytes americanus* (Westin et al., 1979). However, in the north-eastern North Sea *Ammodytes marinus* began maturation at slightly larger sizes—115–120 mm (Bergstad et al., 2001).

Spawned eggs in pacific sand lance are approximately 1.02 mm in diameter, demersal, slightly adhesive, and deposited in the intertidal just below the waterline (Robards et al., 1999a). Similar egg sizes were reported in *Ammodytes marinus* (Gauld and Hutcheon, 1990) and *Ammodytes hexapterus* (Pinto, 1984). In contrast, *Ammodytes americanus* has smaller mature eggs with diameter is 0.6–0.8 mm (Westin et al., 1979).

Similar to many other understudied forage species (e.g., smelts, Burlak and Zhukova, 2020), no previous

studies had previously provided a thorough examination of reproductive maturation processes in pacific sand lance. Detailed metrics related to oogenesis, spermatogenesis, ovarian, and testicular maturity phases are described here for the first time. The detailed morphological criteria provided here describing five distinct histological and macroscopic maturity phases, related to fish length, gonad size and shape, volume occupied by the gonad in the body cavity, and oocyte visibility provide a useful index to categorize and interpret reproductive status, staging and, and capacity in this species.

This study also distinguished size distributions for immature and mature fish and provided minimum estimates for size-at-maturation in males and females. The discovery of bimodal size distributions in mature ovaries, provide strong evidence for group—synchrony ovary organization, determinate fecundity, and the ability to estimate total fecundity prior to the onset of spawning. These are important findings that suggest certain implications for the mechanism, timing, and coordination and synchrony of spawning in these species.

Methods to enable accurate evaluation of reproductive maturation and gonadal development stages are essential to understanding seasonal patterns in reproduction and the onset and duration of spawning in fish populations. This information is also critical to inform analytical stock assessment and estimates of spawning stock abundance. We anticipate that the high-quality maturity data proved in the analyses presented here studies will be useful in informing stock-recruitment relationships and in estimating the effects of parental stock composition on reproductive potential. We also anticipate that this methodology and approach, including the histological description gonadal morphology for members of a particular species or families of fishes might be replicated in other species to similar effect.

ACKNOWLEDGMENTS

COMPLIANCE WITH ETHICAL STANDARDS

REFERENCES

Baker, M.R., Matta, M.E., Beaulieu, M., et al., Intra-seasonal and inter-annual patterns in the demographics of sand lance and response to environmental drivers in the North Pacific, *Mar. Ecol. Prog. Ser.*, 2019, no. 617−618, pp. 221−244.
https://doi.org/10.3354/meps12897

Baker, M.R., Williams, K., Greene, G.H., et al., Use of manned submersible and autonomous stereo-camera array to assess forage fish and associated subtidal habitat, *Fish. Res.*, 2021, no. 243, Article 106067.
https://doi.org/10.1016/j.fishres.2021.106067

Bergstad, O.A., Høines, Å.S., and Krüger-Johnsen, E.M., Spawning time, age and size at maturity, and fecundity of sandeel, *Ammodytes marinus*, in the north-eastern North Sea and in unfished coastal waters off Norway, *Aquat. Liv. Res.*, 2001, vol. 14, no. 5, pp. 293−301.
https://doi.org/10.1016/S0990−7440(01)01134−2

Boulcott, P. and Wright, P.J., Critical timing for reproductive allocation in a capital breeder: evidence from sandeels, *Aquat. Biol.*, 2008, vol. 3, no. 1, pp. 31−40.
https://doi.org/10.3354/ab00063

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., et al., A standardized terminology for describing reproductive development in fishes, *Mar. Coast. Fish.*, 2011, vol. 3, no. 1, pp. 52−70.
https://doi.org/10.1080/19425120.2011.555724

Burlak, O.V. and Zhukova, K.A., Reproductive biology of Rainbow smelt *Osmerus dentex* (*Osmeridae*) of the Amur River, *J. Ichthyol.*, 2020, vol. 60, no. 3, pp. 462−469. https://doi.org/10.1134/S0032945220030054

Chereshnev, I.A., Volobuev, V.V., Khovansky, I.E., and Shestakov, A.V., *Pribrezhnyye ryby severnoy chasti Okhotskogo morya* (Coastal Fishes of the Northern Part of the Sea of Okhotsk), Vladivostok: Dalnauka, 2001.

Ciannelli, L., Winter dormancy in the Pacific Sand Lance (*Ammodytes hexapterus*) in relation to gut evacuation time, in *Forage Fishes in Marine Ecosystems. Proceedings of the Symposium on the Role of Forage Fishes in Marine Ecosystems*, Baxter, B.R., Ed., Fairbanks: Alaska Sea Grant College Program AK−SG−97−01, University of Alaska Fairbanks, 1997.

Doyle, M.J., Picquelle, S.J., Mier, K.L., et al., Larval fish abundance and physical forcing in the Gulf of Alaska, 1981−2003, *Progr. Oceanogr.*, 2009, no. 80, pp. 163−187. https://doi.org/10.1016/j.pocean.2009.03.002

Gauld, J.A. and Hutcheon, J.R., Spawning and fecundity in the lesser sandeel, *Ammodytes marinus Raitt*, in the north-western North Sea, *J. Fish Biol.*, 1990, vol. 36, no. 4, pp. 611−613.

Greene, H.G., Baker, M.R., and Aschoff, J., A dynamic bedforms habitat for the forage fish Pacific sand lance, San Juan Islands, WA USA, in *Seafloor Geomorphology as Benthic Habitat,* Harris, P.T. and Baker, E.K., Eds., New York: Elsevier Sci., 2020. https://doi.org/10.1016/B978−0−12−814960−7.00014−2

Greene, H.G., Baker, M.R., Aschoff, J., and Pacunski, R., Hazards evaluation of a valuable vulnerable sand-wave field forage fish habitat in the marginal Central Salish Sea using a submersible, *Oceanologia*, 2021. https://doi.org/10.1016/j.oceano.2021.06.002

Grier, H.J., Uribe, M.C., Lo Nostro, F.L., et al., Conserved form and function of the germinal epithelium through 500 million years of vertebrate evolution, *J. Morphol.*, 2016, vol. 277, no. 8, pp. 1014−1044. https://doi.org/10.1002/jmor.20554

Lubzens, E., Young, G., Bobe, J., and Cerdà, J., Oogenesis in teleosts: how fish eggs are formed, *Gen. Comp. Endocr.*, 2010, vol. 165, no. 3, pp. 367−389. https://doi.org/10.1016/j.ygcen.2009.05.022

Macer, C.T., Sandeels (*Ammodytidae*) in the south-western North Sea: their biology and fishery, *Fish. Invest., Lond, Ser.*, 1966, vol. 224, no. 6, pp. 1−55.

Matarese, A.C., Blood, D.M., Picquelle, S.J., and Benson, J.L., *Atlas of Abundance and Distribution Patterns of Ichthyoplankton from the Northeast Pacific Ocean and Bering Sea Ecosystems Based on Research Conducted by the Alaska Fisheries Science Center (1972−1996)*, NOAA Prof. Paper 1, 2003.

Murua, H. and Saborido-Rey, F., Female reproductive strategies of marine fish species of the North Atlantic, *J. Northwest Atl. Fish. Sci.*, 2003, no. 33, pp. 23−31. https://doi.org/10.2960/J.v33.a2

Okamoto, H., Sato, H., and Shimazaki, K., Comparison of reproductive cycle between two genetically distinctive groups of sand lance (genus *Ammodytes*) from northern

Hokkaido, *Nippon Suisan Gakkaishi*, 1989, vol. 55, no. 11, pp. 1935−1940.

Orr, J.W., Wildes, S., Kai, Y., et al., Systematics of North Pacific sand lances of the genus *Ammodytes* based on molecular and morphological evidence, with the description of a new species from Japan, *Fish. Bull.*, 2015. vol. 113, pp. 129−156. https://doi.org/10.7755/FB.113.2.3

Pierce, K., Penttila, D., Benson, B., et al., Spatiotemporal detection of foragefish eggs derived from long-term spawning surveys, 2012. http://wdfw.wa.gov/publications/01211/wdfw01211.pdf.

Pinto, J.M., Laboratory spawning of *Ammodytes hexapterus* from the Pacific Coast of North America with a description of its eggs and early larvae, *Copeia*, 1984, vol. 1984, no. 1, pp. 242 −244. https://doi.org/10.2307/1445068

Robards, M.D., Piatt, J.F., and Rose, G.A., Maturation, fecundity, and intertidal spawning of Pacific Sand Lance in the northern Gulf of Alaska, *J. Fish Biol.*, 1999a, vol. 54, pp. 1050−1068. https://doi.org/10.1111/j.1095−8649.1999.tb00857.x

Robards, M.D., Anthony, J.A., Rose, G.A., and Piatt, J.F., Changes in proximate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season, *J. Exp. Mar. Biol. Ecol.*, 1999b, vol. 242, no. 2, pp. 245−258. https://doi.org/10.1016/S0022−0981(99)00102−1

Scott, J.S., Eggs and larvae of northern sand lance (*Ammodytes dubius*) from the Scotian shelf, *J. Fish. Board Canada*, 1972, vol. 29, no. 12, pp. 1667−1671. https://doi.org/10.1139/f72−265

Sisson, N.B., Baker, M.R., Feeding ecology of Pacific sand lance in the San Juan Archipelago, *Mar. Coast. Fish.*, 2017, no. 9, pp. 612−625. https://doi.org/10.1080/19425120.2017.1370043

Tokranov, A.M., Distribution and some biological features of the Pacific sand lance *Ammodytes hexapterus* (Ammodytidae) in waters off Kamchatka in the Sea of Okhotsk, *J. Ichthyol.*, 2007, vol. 47, no. 4, pp. 288−295. https://doi.org/10.1134/S0032945207040054

Uribe, M.C., Grier, H.J., and Mejía-Roa, V., Comparative testicular structure and spermatogenesis in bony fishes, *Spermatogenesis*, 2014, vol. 4, no. 3, Article e983400. https://doi.org/10.4161/21565562.2014.983400

van Deurs, M., Christensen, A., Frisk, C., and Mosegaard, H., Overwintering strategy of sandeel ecotypes from an energy/predation trade-off perspective, *Mar. Ecol. Progr. Ser.*, 2010, vol. 416, pp. 201−214. https://doi.org/10.3354/meps08763

van Pelt, T., Piatt, J., Lance, B., and Roby, D., Proximate composition and energy density of some North Pacific forage fishes, *Compar. Biochem. Physiol.*, 1997, vol. 118, no. 4, pp. 1393−1398. https://doi.org/10.1016/S0300−9629(97)00240−5

Velikanov, A. Ya., Stominok, D. Yu., Occurrence, distribution and some aspects of biology of the Sand Lance (*Ammodytes hexapterus* Pallas, 1811) in the Tatar Strait (Sea of Ja-

pan)), *Izvestiya Tikhookean. Nauchno-Issled. Innst. Rybn. Khoz. Okeanogr.*, 2001, vol. 128, no. 1−3, pp. 737−750.

Westin, D.T., Abernethy, K.J., Meller, L.E., and Rogers, B.A., Some aspects of biology of the American sand lance, *Ammodytes americanus, Trans. Amer. Fish. Soc.*, 1979, vol. 108, no. 3, pp. 328−331.
https://doi.org/10.1577/1548-8659(1979)108<328:SAO-BOT>2.0.CO;2

Whitehouse, G.A., Aydin, K., Essington, T.E., and Hunt, G.L. A trophic mass balance model of the eastern Chukchi Sea with comparisons to other high-latitude systems, *Polar Biol.*, 2014, vol. 37, no. 7, pp. 911−39.
https://doi.org/10.1007/s00300−014−1490−1

Whitehouse, G.A., Aydin, K.Y., Hollowed, A.B., et al., Bottom-up impacts of forecasted climate change on the eastern Bering Sea food web, *Front. Mar. Sci.*, 2021, vol. 8, Article 624301.
https://doi.org/10.3389/fmars.2021.624301

Wilson, M.F., Armstrong, R.H., Robards, M.D., and Piatt, J.F., Sand lance as cornerstone prey for predator populations, in *Sand Lance: A Review of Biology and Predator Relations And Annotated Bibliography,* Robards, M.D., Eds., Portland: U.S. Dept. Agriculture/Forest Service, 1999, pp. 17−44.

Winslade, P.R., Behavioural studies on the lesser sandeel *Ammodytes marinus* (Raitt). III. The effect of temperature on activity and the environmental control of the annual cycle of activity, *J. Fish. Biol.*, 1974, vol. 6, pp. 587−599.
https://doi.org/10.1111/j.1095−8649.1974.tb05102.x