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# Preliminary assessment of a direct age-determination method for 3 commercially important crustaceans from Alaska

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**Abstract**—Management of commercially important crab and shrimp species in Alaska has been hindered by the inability to directly determine the age of individual animals. We investigated the applicability of a recently developed method of age determination to red king crab (*Paralithodes camtschaticus*), southern Tanner crab (*Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*) from Alaska. The cuticle structures of the mesocardiac ossicles of crabs and the eyestalks of spot shrimp were visualized with histological staining to identify the endocuticle, where growth bands have been observed in other crustaceans. For all species, paired light and dark bands were observed in longitudinal, thin sections of these structures in the majority of specimens examined. The proximal portion of the mesocardiac ossicle, where growth bands were observed, was absent in the foregut exuviae of red king and southern Tanner crabs that molted in captivity. If validated, counts of growth bands hold promise as a reliable measure for determining age of these species.

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For many fish and invertebrate species, age can be determined directly from growth bands recorded in calcified hard structures. These structures include bones, scales, and otoliths in fish species (Campana, 2001) and statoliths and shell sections in a variety of invertebrates (Jensen, 1969; Kilada et al., 2007; Abele et al., 2009). Similar methods have not been applied to decapod crustaceans because of the presumed loss and replacement of calcified structures during ecdysis (Vogt, 2012). Instead, indirect methods, including captive observations, tag-recapture experiments, accumulation of lipofuscin in neural tissue, and analysis of size-frequency distributions, have been applied to infer age (Hartnoll, 2001; Vogt, 2012; Pinchuk et al., 2016).

A lack of reliable age information impedes assessment and management of crustacean fisheries (Caddy, 1986). In Alaska, some major crab stocks are assessed and managed by using length-based population models (e.g., Zheng et al., 1995), in which data on abundance, harvest, growth, and mortality are integrated. However, the accuracy of these models may be compromised if the growth or mortality rates are not truly representative of processes in situ. In contrast, age-structured models implicitly account for variability in growth and mortality by incorporating compre-

**Table 1**

Collection location, date, sex, and number of individual red king crab (*Paralithodes camtschaticus*), southern Tanner crab (*Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*) collected in Alaska during 2013–2014 for investigation of presence of growth bands in thin sections of the mesocardiac ossicle of the gastric mill of red king and southern Tanner crabs and in eyestalks of spot shrimp.

| Species | Collection location | Collection date | Sex | *n* |
|---|---|---|---|---|
| Red king crab | Bristol Bay, Alaska | June 2013 | Female | 30 |
| Southern Tanner crab | Marmot Bay, Kodiak, Alaska | June 2014 | Male | 34 |
| Spot shrimp | Seymour Canal, southeast Alaska | February and June 2014 | Male and female | 30 |

hensive size- and abundance-at-age data (Quinn and Deriso, 1999).

A direct method for determining age based on counting bands in the endocuticle layer has been presented for decapod crustaceans (Leland et al., 2011; Kilada et al., 2012). Bands were initially described in the ossicles of the gastric mill for 6 species (Leland et al., 2011; Kilada et al., 2012) and in the eyestalks of 2 additional species (Kilada et al., 2012). These observations have since been extended to additional crab, lobster, and shrimp species and to euphausids (Kilada and Acuna, 2015; Kilada et al., 2015; Sheridan et al., 2015; Kilada and Ibrahim, 2016; Krafft et al., 2016) and have been further supported by technical development, corroboration, and validation (Sheridan et al., 2015; Leland et al., 2015).

Our objective was to evaluate the potential of this method for 3 commercially important species in Alaska: the red king crab (*Paralithodes camtschaticus*), southern Tanner crab (*Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*). Feasibility was evaluated 1) by identifying the endocuticle layer by histological examination, 2) by observing the presence or absence of growth bands in the endocuticle of the mesocardiac ossicles of crabs and eyestalks of shrimp, and 3) by determining whether the region of the mesocardiac ossicle, where growth bands have been observed, may be retained during ecdysis for these crab species. The mesocardiac ossicle was selected as the primary structure for investigation in the 2 crab species because of the presence of clear bands and evidence of possible retention through ecdysis (Leland et al. 2011; Kilada et al., 2012). The eyestalks of shrimp were selected because of the dissimilarity between crab and shrimp gastric mill ossicles and because of the evidence of the presence of bands in the eyestalk of shrimp (Kilada et al., 2012).

## Materials and methods

### Histological examination

Red king crab and spot shrimp were collected in southeast Alaska in 2014, by using pot gear, and southern Tanner crab were collected near Kodiak, Alaska, in 2014 by using trawl gear ($n$=3 for each species). Gastric mills of red king and southern Tanner crabs and paired eyestalks of spot shrimp were dissected and preserved in Bouin's fixative for approximately 1 month and then transported to the Fish Pathology Laboratory of the Alaska Department of Fish and Game in Anchorage, Alaska. Before processing, mesocardiac ossicles of some red king crabs were trimmed to fit within histological cassettes (1 $cm^2$), but most ossicles and eyestalks did not require trimming. Structures were transferred to 70% ethanol and decalcified with Evans and Krajian fluid (Evans and Krajian, 1930). Tissues were dehydrated, infiltrated, and embedded in paraffin with an automatic tissue processor. Histological cassettes were cut longitudinally into 6-µm sections with a rotary microtome, and sections were mounted onto glass slides. Sections of mesocardiac ossicles of crabs and eyestalks of spot shrimp were prepared and stained with Masson's trichrome (Thermo Fisher Scientific[1], Waltham, MA) and permanently mounted with Permount mounting medium (Thermo Fisher Scientific). Masson's trichrome was expected to stain the endocuticle and exocuticle layers blue and the membranous layer (hypodermis) and epicuticle red. The cuticle layers (for detailed description, see Vatcher et al., 2015) were then examined with a Zeiss microscope (Carl Zeiss Microscopy, Jena, Germany) and photographed with a Jenoptik digital camera and ProgRes CapturePro software (JENOPTIK Optical Systems Inc., Jupiter, FL).

### Presence of growth bands

Red king crab, southern Tanner crab, and spot shrimp ($n$=30, 34, and 30, respectively) of a range of body sizes were collected across Alaska in 2013 and 2014 (Table 1). Carapace length (CL) and shell condition of red king crabs, carapace width (CW) and shell condition of southern Tanner crabs, CL of spot shrimp, and sex (identified from pleopod morphological features for shrimp) of all specimens were record-

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

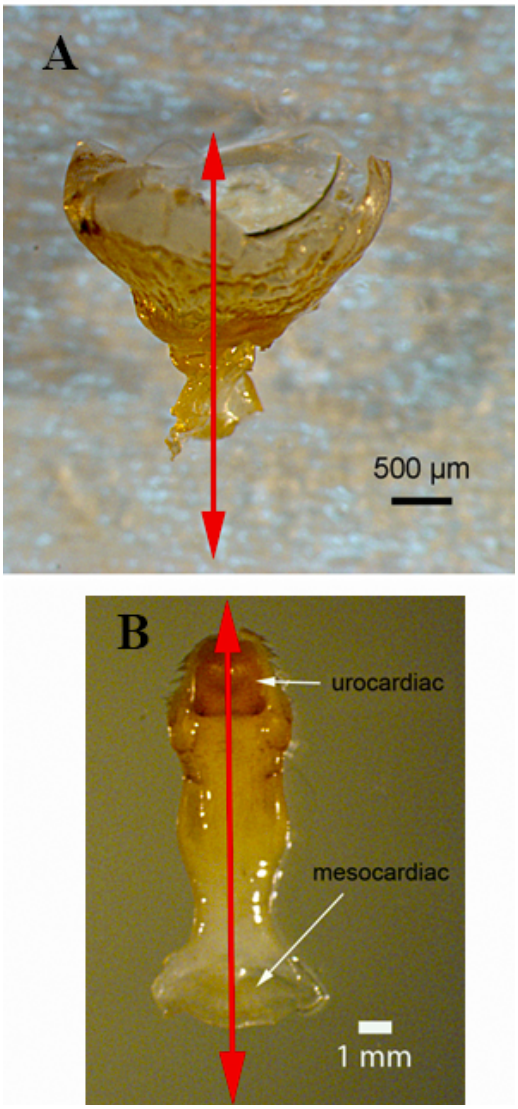


**Figure 1**

Locations of the longitudinal axes used for preparation of thin sections indicated by red arrows for (**A**) the eyestalk of spot shrimp (*Pandalus platyceros*) and (**B**) the dorsal ossicle (urocardiac and mesocardiac) of red king (*Paralithodes camtschaticus*) and southern Tanner (*Chionoecetes bairdi*) crabs, as illustrated with the ossicle of a southern Tanner crab. Red king and southern Tanner crabs and spot shrimp were collected in Alaska during 2013–2014 for this study of a direct age-determination method.

ed at the time of collection. Shell condition, a subjective index of epibionts and wear on the exoskeleton, was noted because it has proven useful for evaluating differences in the recency of molting in crab species (Jadamec et al., 1999; Donaldson and Byersdorfer, 2002). Crab gastric mills and shrimp eyestalks were dissected, cleaned, and preserved in a mixture of ethanol, glycerol, and distilled water (with a volume ratio of 70:4:26).

Structures were embedded in cold cure epoxy resin and sectioned longitudinally (Fig. 1) with a diamond-bladed IsoMet Low Speed Saw (Buehler, Lake Bluff, IL) at the University of New Brunswick in Saint John, New Brunswick, Canada. Several serial sections (with thickness of 160–180 µm) were prepared per structure and mounted with epoxy individually on slides, polished by hand with dry, 0.3-µm-grit lapping film, covered with 90% ethyl alcohol, and viewed under transmitted light with a CX41 Olympus compound microscope (Olympus Corp., Tokyo) at 100–400× magnification. All cuticular layers were examined throughout the thin sections, and bands were recognized as alternating (bipartite) translucent and opaque zones in the endocuticle of crab mesocardiac ossicles and shrimp eyestalks. Photographs were taken with a DP72 Olympus digital camera (Olympus Corp.) attached to the microscope.

### Examination of exuvial gastric structures

Two female red king crab were captured by divers in southeast Alaska and 3 male southern Tanner crab were captured by pot and trawl gear near Kodiak, Alaska, in 2014. Female red king crabs were immature and grasped by males in situ (precopulatory guarding of mate), indicating that the pubertal molt to maturity was imminent. All crabs were held in aquaria with flow-through seawater at ambient seawater temperature and monitored daily until they molted. After ecdysis, the exuvial gastric mill structures were removed and photographed. At 1 week after the molt, the whole stomach was dissected from the crab, and the gastric mill structures were photographed for comparison with the same structures from the exuviae.

## Results

### Histological examination of the cuticle

The endocuticular layer was visually differentiated from other cuticular layers in the mesocardiac ossicles of red king and southern Tanner crabs (Fig. 2) and in the eyestalks of spot shrimp (Fig. 3) after ossicles were stained with Masson's trichrome. The epicuticle, exocuticle, and membranous layers were clearly visible in thin sections of crab mesocardiac, where red indicated the membranous layer and epicuticle (Fig. 2). Lamellar structure, of alternating light and dark stained bands, were more discernible in the mesocardiac of a southern Tanner crab (Fig. 2B) and the exo- and endocuticle of the eyestalk of a spot shrimp (Fig. 3B) than in the mesocardiac of a red king crab (Fig. 2C) at similar magnification. The cuticle layers of the eyestalks of spot shrimp tended to separate during histological sectioning (Fig. 3A).

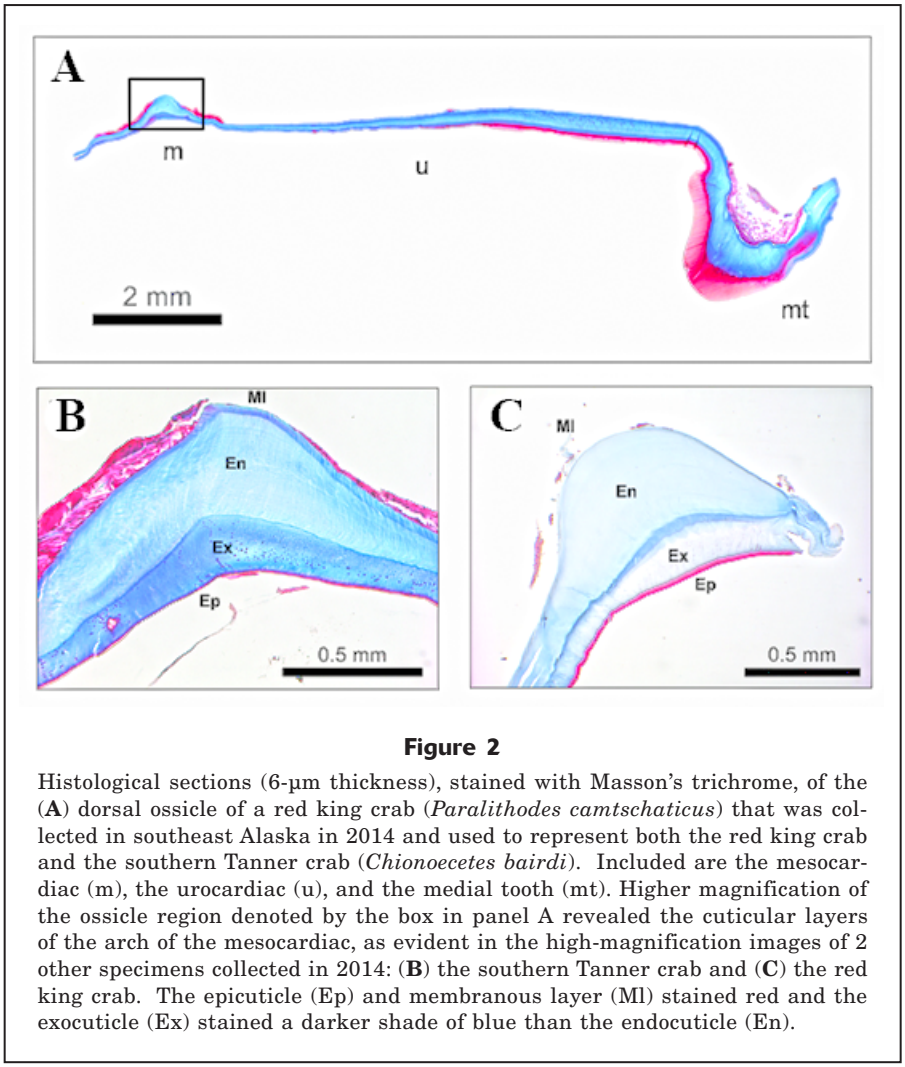


**Figure 2**

Histological sections (6-μm thickness), stained with Masson's trichrome, of the (**A**) dorsal ossicle of a red king crab (*Paralithodes camtschaticus*) that was collected in southeast Alaska in 2014 and used to represent both the red king crab and the southern Tanner crab (*Chionoecetes bairdi*). Included are the mesocardiac (m), the urocardiac (u), and the medial tooth (mt). Higher magnification of the ossicle region denoted by the box in panel A revealed the cuticular layers of the arch of the mesocardiac, as evident in the high-magnification images of 2 other specimens collected in 2014: (**B**) the southern Tanner crab and (**C**) the red king crab. The epicuticle (Ep) and membranous layer (Ml) stained red and the exocuticle (Ex) stained a darker shade of blue than the endocuticle (En).

### Presence of growth bands

Thin sections were successfully obtained from the mesocardiac ossicles from 19 red king crabs and 32 southern Tanner crabs and single eyestalks from 18 spot shrimp. For mesocardiac sections of both crab species, bipartite band patterns (exclusive of the lamellae) were clearest in the location of maximum endocuticle thickness in relation to the exo- and epicuticle that was at the proximal end of the structure (Figs. 2A, 4, A–B). For spot shrimp, bands were clearest at the proximal end of the eyestalk with respect to the anterior tip of the cephalothorax (Figs. 3A, 4C).

### Ossicular retention throughout ecdysis

Comparison of gastric mill structures in exuviae with those of postmolt stomachs of crabs indicated differences in presence of regions of the gastric mill. The cusp of the medial tooth of the urocardiac and zygocardiac ossicles of the gastric mill, along with the portion of the ossicle adjacent to each tooth, were the primary structures present in the exuviae of both southern Tanner and red king crabs (Fig. 5A). For both crab species, the anterior portion of mesocardiac, the pterocardiac, and the anterior portions of the zygocardiac ossicles were not visible in the exuviae, but they were present and robust in a postmolt crab, indicating that these portions were potentially retained or resorbed and subsequently replaced (Fig. 5, B and D).

## Discussion

The presence of bands in the endocuticle layer of the mesocardiacs of southern Tanner and red king crabs and in the eyestalks of spot shrimp indicated that determining age on the basis of band counts may be feasible, as it is for other crustacean species (e.g., Leland et al., 2011; Kilada et al., 2012; Leland et al., 2015; Kilada et al., 2015; Sheridan et al., 2015; Kilada and Ibrahim, 2016; Krafft et al., 2016). Histological charac-

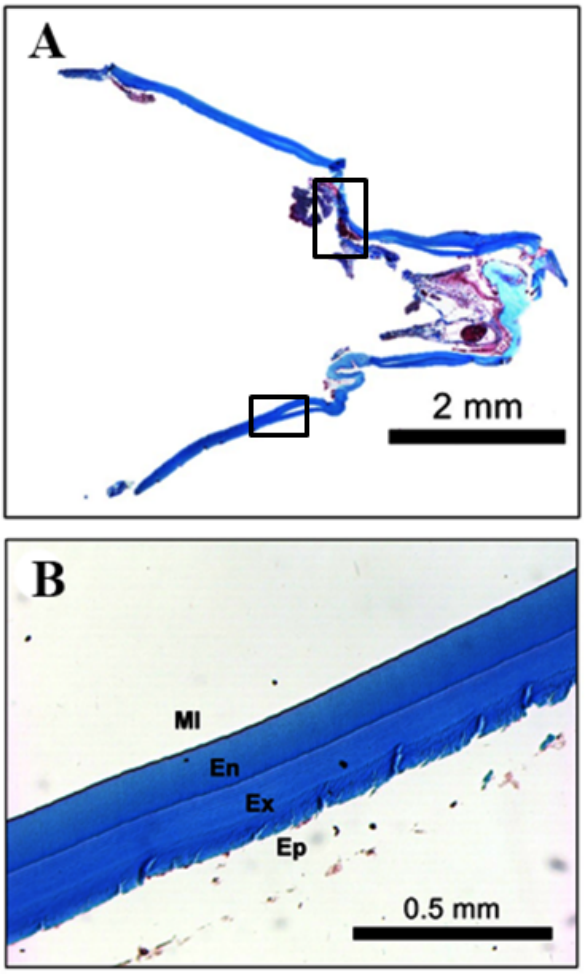


**Figure 3**

Histological sections (6-µm thickness), stained with Masson's trichrome, of the eyestalk of a spot shrimp (*Pandalus platyceros*) collected in Alaska in 2014, showing (**A**) the entire eyestalk and (**B**) the cuticular layering, as seen in the inset in the smaller, lower small box in panel A, including the epicuticle (Ep), exocuticle (Ex), endocuticle (En), and membranous layer (Ml). The larger box indicates the location within the structure where growth bands were observed in Figure 4C.

terization of the mesocardiac ossicles of the crab species and of the eyestalk of the spot shrimp defined the boundaries between cuticular layers—boundaries that are critical for developing band counting criteria. As with results from studies of other species (e.g., Kilada et al., 2012; Leland et al., 2015), there was preliminary evidence that a portion of the mesocardiac ossicle, where band patterns are observed in the endocuticle, is either retained or replaced during molting. A structure that is retained through ecdysis would be useful for evaluating band information.

The cuticle layers of the mesocardiac ossicles of red king and southern Tanner crabs and the eyestalks of spot shrimp were similar to those observed in other studies of decapod crustaceans (see Roer and Dillaman, 1984, and references therein; Vatcher et al., 2015). Differences in staining results may be due to differences in the specific calcium compound involved in the biomineralization within each region (Vatcher et al., 2015). As in results for blue crab (*Callinectes sapidus*) with the use of acridine orange (a different histological stain) (Vatcher et al., 2015), the medial tooth of the urocardiac stained similarly and was continuously connected to the epicuticle in both crab species examined in this study (Fig. 2A). This result further supports the hypothesis that the hardened cusp of the tooth is of epicuticular rather than exocuticular origin (Vatcher et al., 2015).

Bipartite patterns were readily visible in the mesocardiac ossicles of red king and southern Tanner crabs and in the eyestalks of spot shrimp. Recurrence of this pattern in multiple individuals indicates that band counts may be promising as indicators of growth variability through the lifetime of these species. Before this experiment, bands were observed in eyestalks of snow crab (*C. opilio*), which is a congener of the southern Tanner crab (Kilada et al., 2012). We evaluated the mesocardiac of the southern Tanner crab because of the possibility that this structure may be retained through ecdysis (Kilada et al., 2012; Leland et al., 2015; but also see Vatcher et al., 2015). To our knowledge, description of growth bands in red king crabs is a first for the family Lithodidae. The appearance of bands in spot shrimp was very similar to that observed in the eyestalks of a congener, the northern shrimp (*P. borealis*) (Kilada et al., 2012). Finally, most notably for the red king crab, a high proportion of the structures evaluated for growth bands were damaged during embedding or preparing thin sections. Structures were, by necessity, shipped dry before they were embedded in resin, and this condition likely contributed to their fragility and high fracture rate during the embedding and sectioning processes. Embedding structures before shipping could effectively mitigate this problem.

The absence of the basal region of the mesocardiac (where band patterns are observed in thin sections) in exuviae of southern Tanner and red king crabs indicated that this portion of the ossicle was either retained or replaced within 1 week after molting (Fig. 5, A and C). Recently it has been hypothesized that the endocuticle region of the pterocardiac and mesocardiac ossicles is retained through molting (Kilada et al., 2012; Leland et al., 2015). Calcein marks in the endocuticle were visible after several molts and portions of the ossicles were absent in the exuviae of lobster and crayfish species. However, for the blue crab, histological characterization of the cuticular layers of the dorsal ossicle (dorsomedial tooth) indicated that the dorsal cuticle (Roer and Dillaman, 1984), like the endocuticle, is resorbed during the premolt stage and resynthesized during the postmolt stage (Vatcher et al., 2015). As with brachyuran crabs (Brösing, 2014), the gastric teeth differed

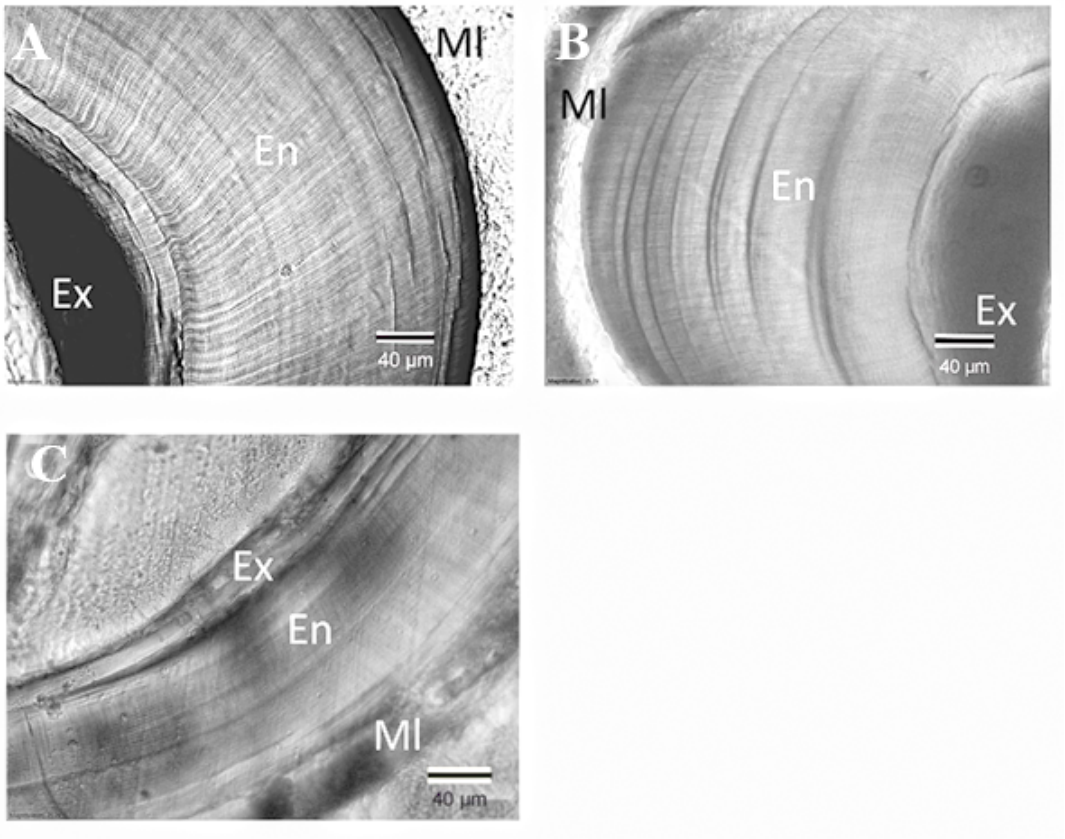


**Figure 4**

Bipartite growth band patterns in the endocuticle of the arch of the mesocardiac ossicle of the gastric mill (for reference, see Fig. 2) of (**A**) a red king crab (*Paralithodes camtschaticus*) collected in Bristol Bay, Alaska, in June 2013; (**B**) a southern Tanner crab (*Chionoecetes bairdi*) collected in Marmot Bay, Alaska, in June 2014; and (**C**) in the proximal portion of the eyestalk of a spot shrimp (*Pandalus platyceros*) collected in Seymour Canal, Alaska, in May 2014, all under transmitted light. The endocuticle (En), exocuticle, (Ex), and membranous layer (Ml) are labeled for orientation.

in biomineralization from other portions of the gastric mill (Vatcher et al., 2015) and remained in the exuviae of both crab species examined in this study.

Our results are an initial step toward developing and further evaluating growth bands as a possible indicator of age for commercially valuable crustaceans in Alaska. Key areas for further research include evaluations of variability (precision) in band count and clarity among the primary ossicles (pterocardiac, zygocardiac, and mesocardiac) of the gastric mill (Leland et al., 2011; Leland et al., 2015; Sheridan et al., 2015), innovation in preparation techniques for thin sections (Sheridan et al., 2015), definitions of criteria for identifying bands (e.g., Leland et al. 2015), determination of the fate (retention or replacement) of the endocuticle during molting (Vatcher et al., 2015), and corroboration of band counts with current understanding of species-specific growth, life history, and longevity based on indirect methods. Ultimately, hypotheses should also be developed regarding the mechanism by which growth bands are formed and retained in structures that are molted (shrimp eyestalk) or possibly retained (e.g., gastric mill ossicles) (Kilada et al., 2012; Leland et al., 2015; Vatcher et al., 2015).

Rigorous validation of bands as indices of age will also be necessary before their application in stock assessment and fisheries management (Beamish and McFarlane, 1983; Campana, 2001; Leland et al., 2011). Validation techniques potentially applicable to the species investigated in this study include the use of autofluorescent stains and the use of specimens with known ages. Autofluorescent stains, such as calcein, can create discrete marks in calcified hard parts that can be used to examine band deposition with

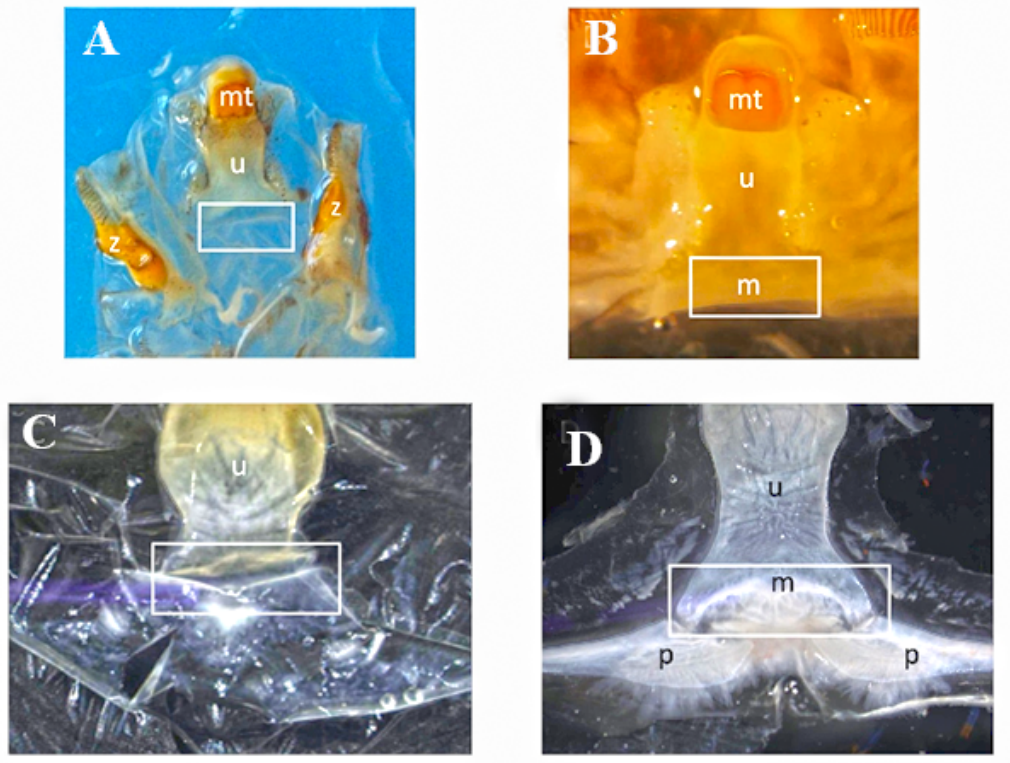


**Figure 5**

(**A**) Image of the primary ossicles of the gastric mill seen in the exuvia of a red king crab (*Paralithodes camtschaticus*) collected in Bristol Bay, Alaska, in June 2013. Included are the lateral teeth of the zygocardiac ossicle (z) and the medial tooth (mt) and urocardiac (u) of the dorsal (uro- and mesocardiac) ossicle, showing that the portion of the ossicle containing the arch of the mesocardiac (m, indicated by the box and illustrated in Fig. 2) was absent. (**B**) Image of the dorsal ossicle of the same red king crab 1 week after ecdysis, showing that the entire mesocardiac was present (boxed). (**C**) The base of the dorsal ossicle for the exuvia of a southern Tanner crab (*Chionoecetes bairdi*) collected in Marmot Bay, Alaska, in June 2014, showing that the urocardiac was present but the mesocardiac (boxed) and pterocardiac ossicles were absent. (**D**) Image of the gastric mill ossicles, showing that the mesocardiac (boxed) and pterocardiac ossicles (p) were present in the ossicle of the southern Tanner crab 1 week after ecdysis.

elapsed time after marking (Kilada et al., 2012; Leland et al., 2015). Further, determining band counts for crustaceans for which ages are known (e.g., animals reared in captivity) will be necessary to further understand dynamics of band formation (Leland et al., 2015).

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