# Realising the reproductive potential of *Parastichopus tremulus* in aquaculture

Gyda Christophersen,[1] Roger Meisal and Jan Sunde


## Abstract

Presently, the sea cucumber *Parastichopus tremulus* is only caught as bycatch from nearshore trawl and pot fisheries because no targeted fishery is allowed in Norway. Due to an increasing market interest, however, the species has been identified as a potential aquaculture candidate. A viable sea cucumber aquaculture production relies on predictable yields of high-quality gametes and the subsequent growth, and survival rates, during the different life stages. Results from initial studies on the reproduction of *P. tremulus* in captivity related to broodstock holding, induced spawning and gamete quality are presented, and new biotechnological tools for improving hatchery production are discussed.


**Keywords:** Norwegian red sea cucumber, *Parastichopus tremulus*, aquaculture, reproduction, gamete quality, genome, fecundity, conditioning

## Introduction

The Norwegian red sea cucumber, *Parastichopus tremulus*, is presently only caught as bycatch from near hore trawl and pot fisheries of fish and crustaceans. No targeted fishery is allowed in Norway unless an environmentally friendly bottom fishing gear can be used. Increased market interest has led to several initiatives with the purpose of exploiting this unused resource. Aquaculture is considered an option to develop a sustainable production of local sea cucumbers, but to reach this level, long-term research and development is needed. *Parastichopus tremulus* lives naturally in the cold temperate waters of the northeast Atlantic Ocean and is also documented from the Mediterranean Sea (Madsen and Hansen 1994; Ordines et al. 2019; Christophersen et al. 2021). Its distribution range is wide, both in terms of latitude (~25–75°N) and depth (20–3000 m) (Grieg 1921; Mortensen 1927; MNHN, Chagnoux S. 2023), suggesting a potential for influencing growth at the different development stages through the modification of environmental conditions and improved culture technology. Knowledge of the reproductive biology of a species is instrumental in designing breeding and aquaculture programmes. Also needed in order to achieve sustainable aquaculture independent of wild animals, is an understanding of the organism's complete life cycle – from spawning to reproductive age in a controlled environment. Predictable reproductive output and growth during the early life stages is crucial to advance the process. The rearing of *P. tremulus* is in its infancy, but spawning and larval production in the lab have successfully been repeated (Christophersen et al. 2020; Christophersen and Sunde 2021; Schagerström et al. 2021). There are many knowledge gaps related to reproduction potential and the possibilities of broodstock conditioning and induced gonad development in culture, for sea cucumbers in general, and for *P. tremulus*, in particular. Broodstock animals for aquaculture may either be collected from the wild within the spawning season, or at other times and maintained in the hatchery under conditions that stimulate gonadal maturation. The spawning season of *P. tremulus* is from June to August, and gonadal development has a clear seasonal pattern (Lönning 1976; Christophersen et al. 2020). The demographic and genetic population structure of *P. tremulus* is not yet known, further emphasising the need for studies related to life history parameters such as stage-specific growth, size at age, and timing of sexual maturity. These events are influenced by environmental and physical conditions. Attempts towards spat production of *P. tremulus* in our lab includes studies on broodstock in captivity, induced spawning, fecundity, and gamete quality. A next step will be the introduction of new biotechological and genetic tools that aid the development of a viable aquaculture industry.

## Broodstock and spawning

Because *Parastichopus tremulus* mainly occurs in waters deeper than 100 m in Norway (Kjerstad et al. 2015), access to live and healthy individuals may be a challenge. We obtain our broodstock sea cucumbers from local fishermen trawling for shrimp (*Pandalus borealis*) or capturing the Norway lobster (*Nephrops norvegicus*) using pots in the fjords nearby Ålesund in western Norway (62°N, 6°E). The sea cucumbers are caught in depths of 100–300 m. Based on our initial work describing the annual reproductive cycle of our local *P. tremulus* where the highest gonad index (GI, % gonad wet weight of body wall wet weight) was registered in May (Christophersen et al. 2020), individuals are brought to the lab in the (Northern Hemisphere's) late spring–early summer for spawning in June and July. A lower GI was found in sea cucumbers kept for a longer time in the lab (from March) compared with individuals obtained shortly before spawning, suggesting that tank conditions influence gonadal maturation (Christophersen et al. 2020). The GI has, over the years, shown large variations between individuals and years, ranging from <0.5% to 23% at peak season.

[1] Møreforsking AS, PO Box 5075, N-6021 Ålesund, Norway. gyda.christophersen@moreforsking.no

In trials comparing the effect of increased temperature, reduced salinity, or air exposure stress (Christophersen et al. 2021), we found that *P. tremulus* was most reliably induced to spawn by a heat shock method. With this method, the sea cucumbers are exposed to a temperature increase of 6°C above the holding temperature for one hour before being transferred to individual spawning containers (Fig. 1). This is also when we can determine the sex without sacrificing the sea cucumbers.

Based on the previously observed GI peak in May (Christophersen et al. 2020), we tried to induce spawning twice weekly, from the end of May to early July 2019 (Table 1). Previous studies have determined the natural spawning time as lasting from July to September in southern Norway (Lönning 1976). We successfully induced spawning in males after temperature stimulation from the end of May, whereas both females and males spawned towards the end of June to the beginning of July (Table 1). From these female spawning events, viable gametes that developed into larvae were produced.

The timing of the collection of broodstock from the wild is important. Liu et al. (2015) stated that it is crucial to collect broodstock when gonads are fully developed because the transfer to new environmental conditions in captivity may arrest the development of immature gonads. We suggest that *P. tremulus* broodstock should be collected no earlier than May. In our lab, spawning trials in subsequent years have confirmed that induced spawning is most successful around the time of midsummer, indicating that the spawning period of our local sea cucumber population is restricted to end of June and July. Sea cucumbers obtained from the sea in August and September may have gonad tissue but are usually in the spent stage or not willing to spawn. The proportion of individuals that actually spawn in captivity varies from season to season, as does the ratio of spawning males to females (Table 2). Thus, the predictability of spawning success in terms of getting large numbers of eggs and synchronous production of egg and sperm is still a gamble, depending on the material that can be obtained by local fishermen.

**Figure 1.** Spawning of *Parastichopus tremulus* in the Møreforsking lab. ©G. Christophersen

**Table 1.** Spawning of *Parastichopus tremulus* at different times during the expected spawning season.

| Date of spawning | No. of individuals | No. of spawned males | No. of spawned females | Percentage spawned |
|---|---|---|---|---|
| 29 May 2019 | 7 | 2 | 0 | 28.6 |
| 31 May 2019 | 7 | 1 | 0 | 14.3 |
| 04 June 2019 | 6 | 1 | 0 | 14.3 |
| 12 June 2019 | 8 | 4 | 0 | 50.0 |
| 18 June 2019 | 4 | 0 | 0 | 0 |
| 24 June 2019 | 12 | 4 | 3 | 58.3 |
| 01 July 2019 | 11 | 4 | 4 | 72.7 |

Table 2. Spawning success of *Parastichopus tremulus* induced to spawn in the lab in different years during the period from 20 June to 3 July.

| | 2017 | 2019 | 2020-1 | 2020-2 | 2021 | 2022-1 | 2022-2 | 2022-3 |
|---|---|---|---|---|---|---|---|---|
| Broodstock group (#) | 34 | 23 | 18 | 22 | 13 | 36 | 21 | 24 |
| Spawned females (#) | 4 | 7 | 4 | 4 | 2 | 6 | 5 | 4 |
| Spawned males (#) | 7 | 8 | 2 | 6 | 7 | 5 | 6 | 0 |
| Spawned (%) | 32 | 65 | 33 | 45 | 69 | 31 | 52 | 17 |

## Broodstock maintenance and conditioning

Worldwide, hatchery production of sea cucumbers is largely based on the collection of broodstock from the wild during times close to the natural spawning period (Agudo 2006; Liu et al. 2015). Successful broodstock maintenance in captivity is needed in order to develop a sustainable aquaculture industry, independent of harvesting sea cucumbers from the wild. Methods of broodstock conditioning – to either enhance the maturation of gonads or to stimulate the rebuilding of spent gonads – will further contribute to predictable and potentially even out-of-season production. The onset and termination of the reproductive period may vary with latitude or from year to year, and is determined by a combination of endogenous and exogenous factors (Giese 1959; Sewell 1992; Mercier and Hamel 2009). Therefore, conditioning regimes must be developed that are specific to species and local stocks. The existing information on methods and conditions for broodstock maturation is limited to a few species, mainly *Holothuria scabra* and *Apostichopus japonicus* (Turner 2015; Hamel et al. 2022). Venâncio et al. (2021) has recently showed the importance of diet during conditioning and how this can be important for larval viability. It has been shown that it is possible to bring *A. japonicus* out of phase by manipulating environmental factors, resulting in a shift in the time of gonadal maturation by up to two months (Liu et al. 2015). From our experience with *P. tremulus*, we know that broodstock can survive in captivity for years, but it remains to see if it is possible to condition them to rebuild or mature their gonads in captivity. Others have faced challenges related to the development of adult sea cucumbers kept in captivity over time, such as reduced growth and fecundity with the number of days in captivity (Morgan 2000a; Venâncio et al. 2021). So far, our attempts to re-spawn broodstock held in captivity have not been too promising, but we believe there is a large room for improvements.

*Parastichopus tremulus* collected for spawning in one season were kept in flow-through holding tanks up to four years as potential broodstock. They were induced to spawn in 2017 and 2019 and kept under conditions with no extra feeding except for the organic matter in the unfiltered incoming seawater from 40 m depth. Trials of re-spawning these individuals in the 2020 and 2021 seasons gave no results in terms of spawning. Poor spawning results were also the case for re-spawning in 2021 of individuals originally taken in for spawning in 2020. In a group given supplemental *Sargassum*-based feed for two months before the attempt to re-spawn, spawning was unsuccessful; whereas in a group that was fed for the entire year resulted in one male spawning. In these preliminary trials, sea cucumbers were not sacrificed to assess gonad status. Accordingly, a new trial was carried out in 2022 with sea cucumbers fed all year and given different conditions from February until the end of June when dissection was performed. Three groups (n=10) were: 1) kept under the standard unfiltered seawater conditions and supplemented with *Sargassum*-based feed enriched with fish feed to increase the protein and lipid content; 2) kept in filtered seawater (1 µm) given *Sargassum* and fish feed; and 3) just *Sargassum*. Mean GI (% ± SD) was low for all groups: 0.68±0.482, 0.09±0.032 and 0.13±0.117 for groups 1, 2 and 3, respectively. In comparison, in 2022 the observed GI in sea cucumbers taken from the wild during the reproductive season was also <1% (0.61±0.683, n=25).

## Fecundity and reproductive potential

The potential reproductive output in terms of gonad size and number of mature eggs varies individually in *P. tremulus* that are caught close to the natural spawning season. Very few broodstock individuals that have been dissected during the natural spawning season have had large amounts of gonad tissue (GI >10%), and the majority has a GI <4%. This could, of course, be due to a loss of gonad tissue during fishing and transport stress, but this is unlikely because we have seldom seen the expulsion of an entire gonad or gonad tissue fragments in our holding tanks. We must also consider that the individuals we have received may or may not be representative samples of the local population distribution of mature and immature sea cucumbers.

To investigate the spawning potential of *P. tremulus* we have made attempts to quantify the fecundity of individuals from induced spawning egg production and from counting remaining oocytes in samples of gonad tissue. Our observations after induced spawning estimate a release of approximately 100–200,000 eggs per female per spawning event (Table 3). This spawning output is probably a fraction of the potential egg production because gonads are not totally emptied during a single spawning event, as has been documented for other sea cucumber species (Sewell 1992; Morgan 2000a; Ramofafia et al. 2000). We have obtained viable eggs from repeated spawnings of the same female (Schagerström et al. 2021). To what extent *P. tremulus* performs multiple batch spawning in nature is unknown. Because we have observed more than one category of gonad developmental stage at the same time (reported in Christophersen et al. 2020), there is a possibility that our sea cucumbers are partial spawners (immature eggs are carried over to the next spawning season), which may complicate the estimation of fecundity.

To quantify the egg production potential, approximately three 0.01 g pieces of filled gonad tissue were sampled from seven females (Table 4). The gonad tissue samples were evaluated under a dissection microscope to confirm they were close to the mature stage. Eggs were separated from the tubules and each sample suspended in 6 mL from where the number of eggs in triplicate volumes of 1 mL was counted. Mean values were used in the calculations of total number of eggs per gonad, and per gram gonad, body wall or total wet weight of the individuals. The number of eggs per gram gonad ranged from 41 to 418,000, averaging 190,000. This is within the same range (50–500,000) as that produced (spawned) by individual *Holothuria scabra* (Morgan 2000a). Based on these estimates, and on the amount of gonad tissue observed in our lab over the years, the proportion of *P. tremulus* females that have the potential of producing millions of eggs seems to be limited, at least for our local population at 62°N. Figure 2 shows the mean number of eggs per gram of total and body wall wet weights. We found lower fecundity of *P. tremulus* than Whitefield and Hardy (2019) reported for *Apostichopus californicus* (≤1000 vs 2863 ± 1502 eggs per g total wet weight).

The relationship between number of eggs counted per gonad correlated well with gonad wet weight (Figure 3), but not with total wet weight of female individuals (R = 0.02; not shown).

## Improving reproduction in captivity

For more developed aquaculture species such as fish, manipulation of environmental parameters and the use of hormonal implants have significantly increased the spawning season in captivity beyond that in nature. The development of these techniques has been essential for establishing profitable industrial aquaculture production of these species. Techniques for efficient reproduction in captivity thus seems crucial to establishing predictable production of juveniles and, thus, secure economic investment to develop industries. Several methods have been developed for other sea cucumbers that could potentially be adapted to improve reproduction of *P. tremulus*. As mentioned previously, common spawning induction protocols are often based on stressing the broodstock temporarily via environmental factors such as raised or lowered temperatures or by exposure to air. However, stress induced by these methods could potentially affect the short- or long-term health of broodstock, and might also be detrimental to the quality of the gametes obtained. Novel biochemical approaches targeting chemical signalling pathways that have been tested in the laboratory but not in aquaculture production systems, may have the potential to be more efficient without exposing the animal to unnecessary stress (see Eeckhaut et al. 2012; Hamel et al. 2022 for reviews). In order to have an industrial application, however, the effects of these compounds need to be tested for confirmation of their properties in each species.

## Gamete quality and storage

When spawning was repeatedly induced in *P. tremulus* males twice a week by temperature shock, sperm initially showed poor motility from the time of the observed GI peak (May–June) (Christophersen et al. 2020). However, from the end of June onwards, quality improved significantly, and collected ejaculates consistently displayed vigorous movement and >90% motile sperm. After collection, sperm motility rapidly degraded and reached 0% within hours, even when stored on ice. From our experience, the relatively short motile period observed, coupled with asynchrony of male and female spawning observed in our laboratory, is a major obstacle to the reliable production of *P. tremulus* larvae and juveniles. Sperm often is either in

Table 3. Number of eggs spawned per female *Parastichopus tremulus*, 2019–2022.

| Year | Female | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2019 | 194,000 | 177,000 | 37,000 | 5000 | |
| 2020 | 82,800 | 101,200 | 240 | 120 | 260 |
| 2022 | 10,1578 | 58,500 | 833 | 3389 | 2400 |

Table 4. Metrics, gonad index and number of eggs (oocytes) in gonad for individual *Parastichopus tremulus*. Egg number is estimated as total number per ovary (average of three samples).

| Individual # | Length (mm) | WW (g) | Body wall WW (g) | Gonad WW (g) | GI (%) | Egg number |
|---|---|---|---|---|---|---|
| **1** | 190 | 273 | 166 | 0.98 | 0.59 | 188,644 |
| **2** | 302 | 356 | 181 | 0.25 | 0.14 | 37,128 |
| **3** | 220 | 221 | 122 | 0.06 | 0.05 | 15,269 |
| **4** | 220 | 383 | 177 | 2.97 | 1.68 | 393,903 |
| **5** | 218 | 238 | 144 | 1.00 | 0.69 | 133,006 |
| **6** | 202 | 200 | 105 | 1.73 | 1.65 | 359,169 |
| **7** | 182 | 259 | 157 | 2.24 | 1.42 | 577,508 |

WW: wet weight

excess or lacking, depending on the success rate and gender distribution of broodstock induced to spawn. However, sea cucumber sperm seemingly can be used for *in vitro* fertilisation even when harvested from dissected gonads (Shao et al. 2006). This has not yet been attempted for *P. tremulus*. Even though this technique could provide more reliable access to sperm for fertilisation and possibly reduce the total number of males needed, we do not consider this a sustainable model for hatchery production, because it requires sacrificing potentially, genetically valuable males. Development of additional methods that facilitate storage of gametes, both in the short or long term, is necessary to improve the efficiency of reproduction in captivity.

As with other externally fertilising marine organisms, sperm motility in holothuroids is initiated by changes in osmolarity upon release to the surrounding seawater (Yu et al. 2011). Motility can also be modulated by changes in pH, salinity and concentration of specific ions, as demonstrated in *Apostichopus japonicus* (Shao et al. 2006; Yu et al. 2011). Methods for short-term storage of sperm from other species have taken advantage of these properties to successfully develop buffer solutions that inhibit motility and prolongs the motile period and fertilising ability of the sperm. Although some progress has been made with regards to storage solutions for sperm from other echinoderm species (see review by Gwo 2000), little has been published on sea cucumber sperm storage. Sperm motility inhibition and re-activation has been demonstrated for *A. japonicus* sperm collected from gonads (Shao et al. 2006; Yu et al. 2011), and without any additional treatment, sperm excised from gonads could be stored in their original seminal fluid at 4°C and still be activated after one day (Shao et al. 2006). A cryopreservation protocol has been developed for *A. japonicus* sperm that is both simple and effective for hatchery applications (Shao et al. 2006; Xu et al. 2022) and, at present, seems to be the best option for the storage of *P. tremulus* sperm. Hopefully, more research efforts will be focused on adapting this method to new species, or developing new cryopreservation protocols. Sperm cryopreservation is not only a useful tool for aquaculture purposes, but has a particular important application to conservation of genetic resources from endangered sea cucumber stocks worldwide. In contrast to sperm, oocytes from dissected gonads of

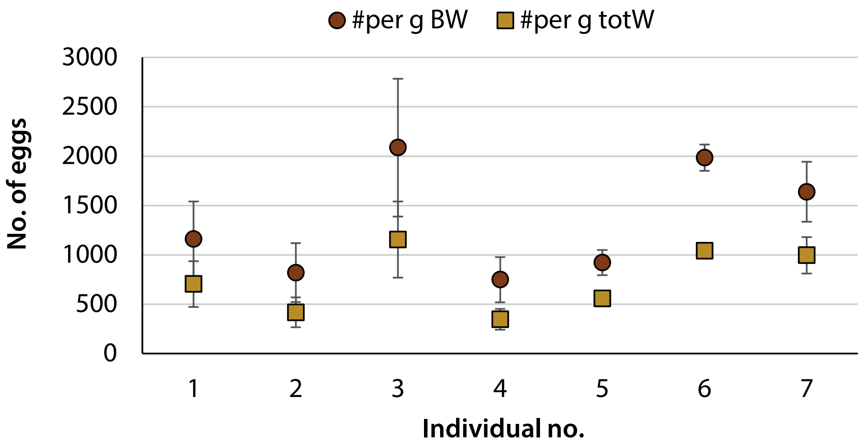


**Figure 2.** For the seven individuals, the average number of eggs (± SD) in gonads per gram of body wall and total wet weight of *Parastichopus tremulus*.

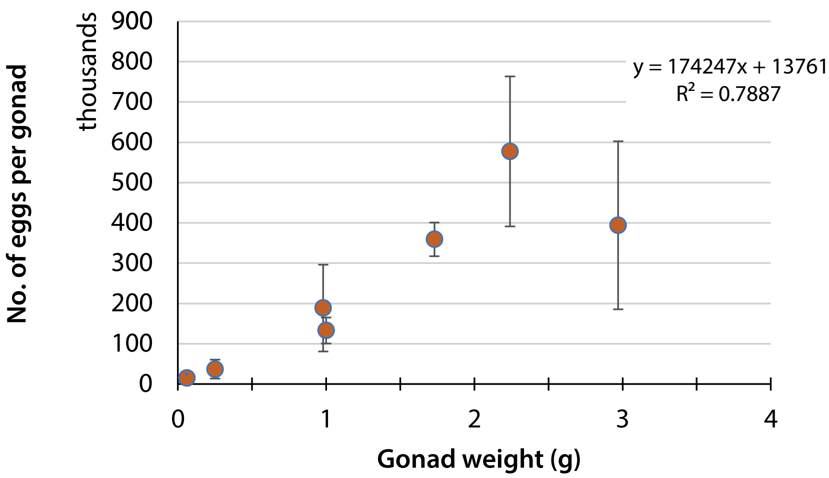


**Figure 3.** Relationship between gonad wet weight of seven individuals and the average number of eggs per gonad (± SD) in mature *Parastichopus tremulus*.

females can rarely be fertilised *in vitro*, although some promising efforts have been made towards achieving final oocyte maturation *in vitro* (see Léonet et al. 2009, 2019; Hamel et al. 2022; Eeckhaut et al. 2012 for reviews). So far, no trials have been carried out on *P. tremulus* oocytes.

We can speculate as to whether all sexually mature individuals of *P. tremulus* reproduce annually, or if gonad maturation happens over years, such as that described in the proposed tubule recruitment model of gonad development for *Apostichopus californicus* (Smiley and Cloney 1985). This model, however, does not seem to be applicable to all holothuroid species because gonad development may be different between closely related species and even within geographically separated populations of the same species (Sewell et al. 1997; Hamel et al. 2022). It has also been observed that variations in environmental factors from year to year impact the gonad maturation processes (Sewell and Bergquist 1990; Sewell et al. 1992). More thorough investigations into gonad development in *P. tremulus* over several years needs to be performed in order to gain a better understanding of this process and how it can be manipulated in captivity.

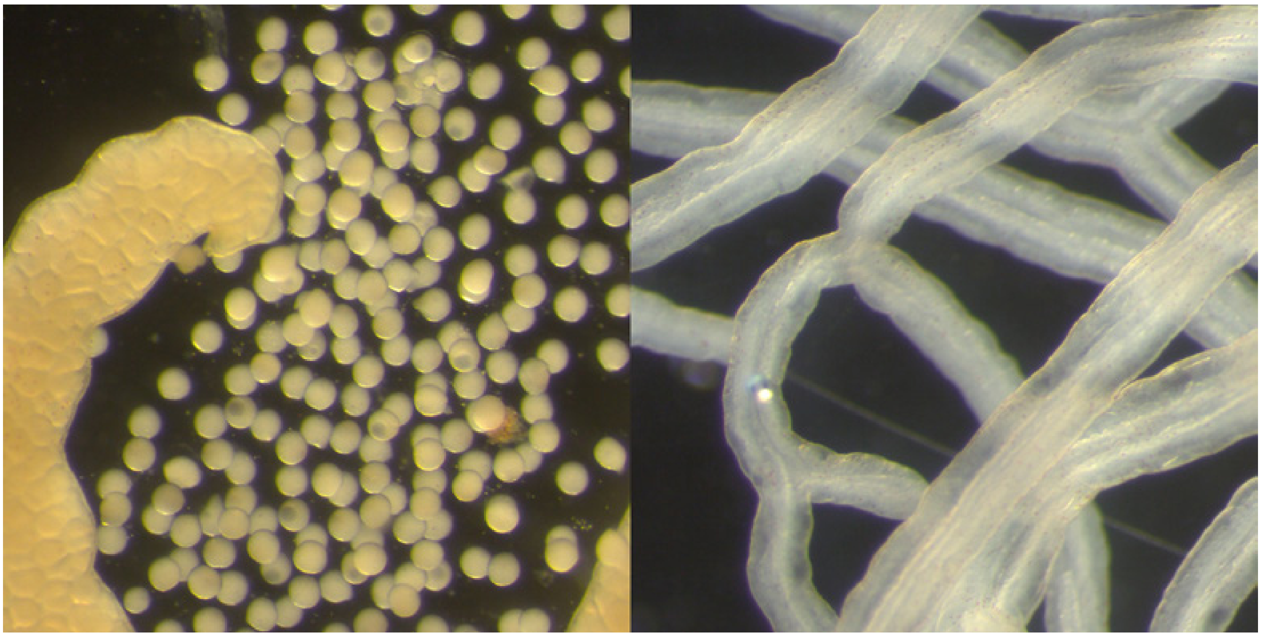

**Figure 4.** Female (left) and male (right) gonads of *Parastichopus tremulus*. @ G. Christophersen

## Genome and genomic tools

Knowledge of the sequence, structure and genetic content of animal genomes is being used at an increasingly larger scale in breeding programmes to select for traits for improved animal health and to increase production (Rexroad et al. 2019). The first sea cucumber genome to be published was of *Apostichopus parvimensis* in February 2015. To date, 22 nuclear genomes of 21 different sea cucumber species are available at the National Center for Biotechnology Information (NCBI) in the database Genbank (search term "Holothuroidea"), including colour variants of *Apostichopus japonicus*, the most studied species (Jo et al. 2017; Zhang et al. 2017). None of the nuclear genomes are currently in NCBI RefSeq (a non-redundant database collection of reference sequences). However, of the 109 currently available mitochondrial genomes, encompassing 46 verified species, 29 can be found in RefSeq. Research interest in genome data from sea cucumbers seems to be accelerating because 18 of the nuclear genomes were submitted and or published between January 2021 and February 2023. There are currently no genome data available for *P. tremulus*. Only a few mitochondrial gene fragments such as the Cytochrome Oxidase subunit I (COI) that are typically used for species identification of eukaryotes are available in Genbank (https://www.ncbi.nlm.nih.gov) (Ratnasingham and Hebert 2007).

Thus far, the genome sequence of *Stichopus chloronotus* (unpublished direct submission, assembly accession GCA_021234535) is the only one that has achieved chromosome resolution on its assembly, all other sea cucumber assemblies are in the form of contigs or scaffolds and are, thus, considered draft genomes. This is also the case in general for most other genomes assembled today. Although there may be both inter- and intra-species variation in both ploidity and chromosome number in Holothuroidea, genomes are most consistently reported to be diploid, with 22 chromosome pairs (Colombera 1974; Okumura et al. 2008; Zhang et al. 2017). DNA sequencing technology is developing at a fast rate and has gone from being available exclusively at larger sequencing facilities, to currently being available in most research laboratories and even in the field. To answer some of the pressing questions regarding aspects of the biology and function of the red sea cucumber, Møreforsking has initiated work to sequence, assemble, and annotate the whole genome of *P. tremulus* using Nanopore sequencing (Oxford Nanopore Technology, Oxford, United Kingdom). The resulting genome will improve our understanding of this species and aid in the development of farming and breeding technologies, as well as enable bioprospecting of high-value compounds. So far, with the currently produced *P. tremulus* DNA sequence data, a low-quality assembly has been produced (Meisal, unpublished).

These data can already be used to search for answers to questions relating to the reproduction of *P. tremulus*. Due to the lack of external gender-specific characteristics in *P. tremulus*, determining the gender of potential broodstock before spawning is difficult. Techniques for gender identification could be developed based on *e.g.* gonad biopsy (Morgan 2000b; Pratas et al. 2017), but identification of gender-specific genes as previously described for *Holothuria scabra* and *Apostichopus japonicus* (Zixuan et al. 2023; Wei et al. 2021) would potentially be a good option when a reliable and inexpensive method becomes available.

## Future possibilities to realise the reproductive potential

Reliable methods for spawning induction and gamete storage for *P. tremulus* are still being developed, but several promising strategies can be pursued to adapt protocols developed for other species for spawning induction, oocyte maturation and cryopreservation of sperm. By advancing the methods of conditioning to successfully manipulate maturation of gonads in captivity, opportunities lie ahead for the synchronous maturation and reliable spawning of males and females. This in turn will enhance larval yield, a prerequisite for predictable juvenile production.

In parallel to optimising methods for broodstock conditioning and reproduction, our ambition in the near future is to assemble the first complete *P. tremulus* genome. This will contribute to the relatively scarce information on holothuroid genetics, and hopefully lead to increased knowledge that will assist the development of new tools for aquaculture of emerging sea cucumber species.

## Acknowledgement

The work has been funded by the Research Council of Norway – SANOCEAN (project no. 288536), and the Møre og Romsdal County Municipality Post-Doc. R. Meisal (project no. 2021-0165).

## References

Agudo N. 2006. Sandfish hatchery techniques. New Caledonia: Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and the WorldFish Center. https://purl.org/spc/digilib/doc/ze7r8

Christophersen G. and Sunde J. 2021. Norwegian red sea cucumber (*Parastichopus tremulus*). Steps towards life in captivity – a viable option? Poster – Aquaculture Europe 2020, April 2021. DOI: 10.13140/RG.2.2.31943.83366

Christophersen G., Bakke S. and Sunde J. 2021. Norwegian red sea cucumber (*Parastichopus tremulus*) fishery and aquaculture north of 60°N latitude: Feasible or fictional? SPC Beche-de-mer Information Bulletin 41:25–36. https://purl.org/spc/digilib/doc/fvfxj

Christophersen G., Bjørkevoll I., Bakke S. and Kjerstad M. 2020. Reproductive cycle of the red sea cucumber, *Parastichopus tremulus* (Gunnerus, 1767), from western Norway. Marine Biology Research 16(6–7):423–430.

Colombera D. 1974. Chromosome evolution in the phylum Echinodermata. Journal of Zoological Systematics and Evolutionary Research 12(1):299–308. https://doi.org/10.1111/J.1439-0469.1974.TB00172.X

Eeckhaut, I., Lavitra T., Leonet A., Jangoux M. and Rasolofonirina R. 2012. In-vitro fertilisation: A simple, efficient method for obtaining sea cucumber larvae year round. p. 40–49. In: Hair C.A., Pickering T.D. and Mills D.J. (eds). Asia-Pacific Tropical Sea Cucumber Aquaculture. Proceedings of an International Symposium, Noumea, New Caledonia. ACIAR Proceedings.

Giese A.C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Annual Review of Physiology 21(1):547–576.

Grieg J.A. 1921. Echinodermata. Trustees of the Bergen Museum. In: Report of the scientific results of the Michael Sars North Atlantic Deep Sea Expedition 1910. Bergen, Norway: Trustees of the Bergen Museum, vol. III, part 2.

Gwo J.C. 2000. Cryopreservation of aquatic invertebrate semen: a review. Aquaculture Research 31:259–271. https://doi.org/10Gwo.1046/j.1365-2109.2000.00462.x

Hamel J.F., Eeckhaut I., Conand C., Sun J., Caulier G. and Mercier A. 2022. Global knowledge on the commercial sea cucumber *Holothuria scabra*. p. 1–286. In: Advances in Marine Biology vol. 91. Cambridge, USA: Academic Press.

Jo J., Oh J., Lee HG., Hong H.H., Lee S.G., Cheon S., Kern E.M.A., Jin S., Cho S.J., Park J.K. and Park C. 2017. Draft genome of the sea cucumber *Apostichopus japonicus* and genetic polymorphism among color variants. Gigascience 6(1):1–6.

Kjerstad M., Ringvold H., Søvik G., Knott E.K. and Thangstad T. H. 2015. Preliminary study on the utilisation of Norwegian red sea cucumber, *Parastichopus tremulus* (Gunnerus, 1767) (Holothuroidea, Echinodermata), from Norwegian waters: Resource, biology and market. p. 109–132. In: Gundersen A.C. and Velle L.G. (eds). Orkana Akademisk, Norway: Blue Bio-resources.

Léonet A., Rasolofonirina R., Wattiez R., Jangoux M. and Eeckhaut I. 2009. A new method to induce oocyte maturation in holothuroids (Echinodermata). Invertebrate Reproduction and Development 53(1):13–21. https://doi.org/10.1080/07924259.2009.9652285

Léonet A., Delroisse J., Schuddinck C., Wattiez R., Jangoux M. and Eeckhaut I. 2019. Thioredoxins induce oocyte maturation in holothuroids (Echinodermata). Aquaculture 510:293–301. https://www.sciencedirect.com/science/article/abs/pii/S0044848618304459?via%3Dihub

Liu S., Sun J., Ru X., Hamel J.-F. and Mercier A. 2015. Chapter 7. Broodstock conditioning and spawning. p. 101–110. In: Yang H., Hamel J.-F. and Mercier A. (eds). The sea cucumber *Apostichopus japonicus*, history, biology and aquaculture. Cambridge, USA: Academic Press. https://www.sciencedirect.com/science/article/abs/pii/B9780127999531000076?via%3Dihub

Lönning S.1976. Reproductive cycle and ultrastructure of yolk development in some echinoderms from the Bergen area, western Norway. Sarsia 6281:49–72.

Madsen F.J. and Hansen B. 1994. Echinodermata, Holothurioidea. Marine Invertebrates of Scandinavia Number 9. Oslo: Scandinavian University Press. 143 pp.

Mercier A. and Hamel J.F. 2009. Endogenous and exogenous control of gametogenesis and spawning in echinoderms. Advances in Marine Biology 55:1–6.

MNHN, Chagnoux S 2023. The echinoderm collection (IE) of the Muséum national d'Histoire naturelle (MNHN – Paris). Version 75.297. MNHN – Museum national d'Histoire naturelle. Occurrence dataset https://doi.org/10.15468/tp2nxo accessed via GBIF.org on 2023-02-02.

Morgan A.D. 2000a. Induction of spawning in the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea). Journal of the World Aquaculture Society 31(2):186–194.

Morgan A.D. 2000b. Aspects of sea cucumber broodstock management (Echinodermata: Holothuroidea). SPC Beche-de-mer Information Bulletin 13:2–8. https://purl.org/spc/digilib/doc/i47y9

Mortensen T. 1927. Handbook of the Echinoderms of the British Isles. Oxford, UK: Oxford University Press.

Okumura S.I., Kimura K., Sakai M., Waragaya T., Furukawa S., Takahashi A. and Yamamori K. 2008. Chromosome number and telomere sequence mapping of the Japanese sea cucumber *Apostichopus japonicus*. Fisheries Science 75(1):249–251. http://doi.org/10.1007/s12562-008-0025-5

Ordines F., Ferriol P., Moya-Ruiz F., Farias C., Rueda J.L. and Garcia-Ruiz C. 2019. First record of the sea cucumber *Parastichopus tremulus* Gunnerus, 1767 (Echinodermata: Holothuroidea: Aspidochirotida) in the Mediterranean Sea (Alboran Sea, western Mediterranean). Cahiers de Biologie Marine 60:111–115. doi: 10.21411/CBM.A.137C121D

Pratas D., Santos F., Dias F., Rodrigues V., Couto M., Santos R., Baptista T. and Pombo A. 2017. Development of techniques for gender identification in *Holothuria forskali* (Delle Chiaje, 1823). SPC Beche-de-Mer Information Bulletin 37:95–98.

Ramofafia C., Battaglene S.C., Bell J.D. and Byrne M. 2000. Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. Marine Biology 136(6):1045–1056.

Ratnasingham S. and Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7.3:355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rexroad C., Vallet J., Matukumalli L.K., Reecy J., Bickhart D., Blackburn H., Boggess M, Cheng H., Clutter A., Cockett N., Ernst C., Fulton J.E., Liu J., Lunney J., Neibergs H., Purcell C., Smith T.P.L., Sonstegard T., Taylor J., Telugu B., Eenennaam A.V., Tassell C.P.V. and Wells K. 2019. Genome to phenome: Improving animal health, production, and well-being – A new USDA blueprint for animal genome research 2018–2027. Frontier in Genetics 10:327. https://doi.org/10.3389/fgene.2019.00327

Schagerström E., Christophersen G., Sunde J., Bakke S., Matusse N.R., Dupont S. and Sundell K.S. 2021. Controlled spawning and rearing of the sea cucumber, *Parastichopus tremulus*. Journal of the World Aquaculture Society 53(1):224–240. https://doi.org/10.1111/jwas.12816

Sewell M.A. 1992. Reproduction of the temperate aspidochirote *Stichopus mollis* (Echinodermata: Holothuroidea) in New Zealand. Ophelia 35(2):103–121.

Sewell M.A. and Bergquist P.R. 1990. Variability in the reproductive cycle of *Stichopus mollis* (Echinodermata: Holothuroidea). Invertebrate Reproduction and Development 17(1):1–7. https://doi.org/10.1080/07924259.1990.9672081

Sewell M. A., Tyler P. A., Young C. M. and Conand C. 1997. Ovarian Development in the Class Holothuroidea: a Reassessment of the "Tubule Recruitment Model". The Biological Bulletin 192(1):17-26. https://doi.org/10.2307/1542572

Shao M.Y., Zhang Z.F., Yu L., Hu J.J. and Kang K.H. 2006. Cryopreservation of sea cucumber *Apostichopus japonicus* (Selenka) sperm. Aquaculture Research 37(14):1450–1457. https://doi.org/10.1111/j.1365-2109.2006.01581.x

Smiley S. and Cloney R.A. 1985. Ovulation and the fine structure of the *Stichopus californicus* (Echinodermata: Holothuroidea) fecund ovarian tubules. Biological Bulletin 169(2):342–364. https://doi.org/10.2307/1541487

Turner L.H. 2015. Reproductive conditioning and spawning of the sea cucumber *Holothuria scabra* for hatchery production. PhD thesis, University of the Sunshine Coast, Queensland, Australia.

Venâncio E., Félix P.M., Brito A.C., Sousa J., Azevedo e Silva F., Simões T., Narciso L., Amorim A., Dâmaso L. and Pombo A. 2021. Do broodstock diets influence viability and larval development of *Holothuria mammata*? Aquaculture 536:736431. https://doi.org/10.1016/j.aquaculture.2021.736431

Wei J.L., Cong J.J., Sun Z.H., Song J., Zhao C. and Chang Y.Q. 2021. A rapid and reliable method for genetic sex identification in sea cucumber, *Apostichopus japonicus*. Aquaculture 543 (2021) 737021. https://doi.org/10.1016/j.aquaculture.2021.737021

Whitefield C.R. and Hardy S.M. 2019. Estimates of reproductive potential and timing in California sea cucumbers *Parastichopus californicus* (Stimpson, 1857) from southeast Alaska based on natural spawning. Journal of Shellfish Research 38(1):191–199.

Xu S., Liu S., Sun J., Zhang L., Lin C., Sun L., Xing L., Jiang C. and Yang H. 2022. Optimizing cryopreservation of sea cucumber (*Apostichopus japonicus*) sperm using a programmable freezer and computer-assisted sperm analysis. Frontiers in Marine Science 9:1–17. https://doi.org/10.3389/fmars.2022.917045

Yu L., Shao M., Bao Z., Hu J. and Zhang Z. 2011. Effects of environment factors on initiation of sperm motility in sea cucumber *Apostichopus japonicus* (Selenka). Journal of Ocean University of China 10(2):165–169. https://doi.org/10.1007/s11802-011-1748-y

Zhang X., Sun L., Yuan J., Sun Y., Gao Y., Zhang L., Li S., Dai H., Hame J.F.Q., Liu C., Yu Y., Liu S., Lin W., Guo K., Jin S., Xu P., Storey K.B., Huan P., Zhang T., Zhou Y., Zhang J., Lin C., Li X., Xing L., Huo D., Sun M., Wang L., Mercier A., Li F., Yang H. and Xiang J. 2017. The sea cucumber genome provides insights into morphological evolution and visceral regeneration. PLoS Biology 15(10):e2003790. https://doi.org/10.1371/journal.pbio.2003790

Zixuan E., Cheng C., Wu F., Ren C., Chen R., Rao Y., Ma B., Jiang X., Luo P., Li X., Zhang X., Jiang F., Hu C. and Chen T. 2023. Nondestructive and rapid method for sex identification of the tropical sea cucumber *Holothuria scabra* by anal swab sampling. Aquaculture 562: 738749. https://doi.org/10.1016/j.aquaculture.2022.738749