been completely sequenced to date, estimates are that the American lobster (*Homarus americanus*) genome is about 4.40 pg/4.3 Gbp or greater (see Animal Genome Size Database website and Gloucester Marine Genomics Institute website, American Lobster Genome, on the internet).

[007] The lack of information regarding shrimp, prawn, crab, crayfish, and/or lobster is arguably largely due to challenges in cell culture. For example, shrimp/prawn cell lines have only been established from the lymphoid organ and ovaries (Tapay *et al*. (1995) Proc Soc Exp Biol Med 209(1):73-8; Hsu *et al*. (1995) Aquaculture 136:43-55; US patent number 6143547; George and Dhar (2010) In Vitro Cell Dev Biol Anim 46(9):801-10; Ma *et al*. (2017) Reviews in Aquaculture 9: 88-98). In addition, shrimp/prawn primary muscle cultures have lasted only up to 12 days (George and Dhar, 2010) and no known primary cultures or cell lines have been described for fat cells.

[008] Furthermore, attempts to culture fat cells from shrimp, prawn, crab, crayfish, and/or lobster have not been described.

[009] The availability of immortalized cell lines from shrimp, prawn, crab, crayfish, and/or lobster would allow cellular agriculture companies to progress to the next steps of cellular agriculture (Rubio *et al*., (2019) Front. Sustain. Food Syst. doi: 10.3389/fsufs.2019.00043). Rather than the time consuming process of isolating primary cells, food regulatory and research groups would then have a ready source of cell lines. This would facilitate understanding shrimp, prawn, crab, crayfish, and/or lobster viral diseases, testing for the presence of those viruses, and developing treatments (Ma *et al*. (2017) Reviews in Aquaculture 9: 88-98). Consequently, there remains a need for immortalized muscle and fat cell lines from shrimp, prawn, crab, crayfish, and/or lobster. The disclosure presented herein addresses this need.

SUMMARY

[009a] In one aspect, the present disclosure refers to a renewable crustacean cell line comprising primary muscle cells.

[009b] In another aspect, the present disclosure refers to a crustacean tissue comprising the

renewable crustacean muscle cell line as described herein.

[009c] In another aspect, the present disclosure refers to a meat product comprising the renewable crustacean muscle cell line as described herein.

[009d] In another aspect, the present disclosure refers to a method for producing a shrimp, prawn, crab, crayfish, and/or lobster renewable muscle cell line as described herein comprising (a) isolating shrimp, prawn, crab, crayfish, and/or lobster muscle tissue; (b) sterilizing the isolated muscle tissue and optionally removing fat and epidermal tissue; (c) mincing the sterilized isolated muscle tissue and placing the minced sections into cell culture media; (d) incubating the media and minced sections of step (c) at 28^{o}C without CO_2 for 3 to 7 days; (e) removing the remaining minced sections, adding fresh media, and incubating the remaining cells in the media at 28^{o}C without CO_2 for 3 to 7 days; and (f) repeating step (e) when cells number about 8 million in 10 ml of culture media, wherein step (f) is repeated at least 10 times.

[009e] In another aspect, the present disclosure refers to cell cultures comprising the renewable crustacean muscle cell lines as described herein.

[0009f] In another aspect, the present disclosure refers to a cell culture comprising the renewable crustacean muscle cell line as described herein, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus*.

[009g] In yet another aspect, the present disclosure refers to a cell culture comprising the renewable crustacean muscle cell line as described herein, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells shrimp, and/or prawn muscle cells are from *Penaeus monodon* and/or *Litopenaeus vannamei*.

[010] The present disclosure is directed to tissue engineered shrimp, prawn, crab, crayfish, and/or lobster meat products and methods for producing such products. In one example, the meat product comprises muscle cells that are grown *ex vivo* and/or *in vitro*. In a further example, the

meat product comprises muscle and/or fat cells grown *ex vivo* and/or *in vitro*. In yet another example, the meat product comprises muscle cells, fat cells, other cells, and combinations thereof *grown ex vivo and/or in vitro*. The meat product is essentially free of any harmful microbial or parasitic contamination.

[011] Accordingly, the present disclosure also provides methods of producing shrimp, prawn, crab, crayfish, and/or lobster meat products for consumption by developing renewable muscle and/or fat stem cell lines.

[012] One method for producing a shrimp, prawn, crab, crayfish, and/or lobster immortalized muscle and/or fat cell line includes isolating shrimp, prawn, crab, crayfish, and/or lobster muscle and/or fat cells, integrating at least one muscle-specific and/or fat-specific growth gene into an adenoviral vector or non-integrative lentiviral vector to form a recombinant adenoviral construct or non-integrative lentiviral vector and transducing the isolated shrimp, prawn, crab, crayfish, and/or lobster muscle cells with the recombinant adenoviral construct or non-integrative lentiviral vector. However, the method for producing a shrimp, prawn, crab, crayfish, and/or lobster immortalized muscle and/or fat cell line also includes inducing activity of telomerase after isolation of shrimp, prawn, crab, crayfish, and/or lobster muscle and/or fat cells by chemical activators such as Cycloastragenol, Genistein, and/or Resveratrol.

[013] Also presented is a method for producing shrimp, prawn, crab, crayfish, and/or lobster induced pluripotent stem (iPS) cells. This method includes isolating muscle and/or fat cells from shrimp, prawn, crab, crayfish, and/or lobster, transfecting the cells with an episomal plasmid comprising a targeting factor and then culturing the transfected cells to produce iPS cells.

[014] In addition, the present disclosure provides a method for producing shrimp, prawn, crab, crayfish, and/or lobster induced pluripotent stem (iPS) cells via isolating shrimp, prawn, crab, crayfish, and/or lobster muscle and/or fat cells, transfecting the cells with microRNA containing reprogramming microRNAs and mRNAs such as mir302a-d, mir367, Oct4, Sox2, Klf4, c-Myc, and Lin28, and culturing the transfected cells to produce iPS cells.

CLAIMS

We claim

1. A renewable crustacean cell line comprising primary muscle cells.

2. The renewable crustacean cell line according to claim 1, wherein the cell line has completed at least 10 passages.

3. The renewable crustacean cell line according to claim 1, wherein the primary muscle cells are stem cells.

4. The renewable crustacean cell line according to claim 1, wherein the primary muscle cells differentiate into myofibers.

5. The renewable crustacean muscle cell line of claim 1, wherein the primary muscle cells are immortalized primary muscle cells.

6. The renewable crustacean muscle cell line of claim 1, wherein the primary muscle cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.

7. The renewable crustacean muscle cell line of claim 1, wherein the primary muscle cells are shrimp or lobster primary muscle cells.

8. A crustacean tissue comprising the renewable crustacean muscle cell line of claim 1.

9. A meat product comprising the renewable crustacean muscle cell line of claim 1.

10. The meat product according to claim 9 , further comprising other shrimp, prawn, crab, crayfish, and/or lobster cells.

11. The meat product according to claim 9, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus.*

12. The meat product according to claim 9, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells from *Penaeus monodon* and/or *Litopenaeus vannamei*.

13. A method for producing a shrimp, prawn, crab, crayfish, and/or lobster renewable muscle cell line according to claim 1 comprising

(a) isolating shrimp, prawn, crab, crayfish, and/or lobster muscle tissue;

(b) sterilizing the isolated muscle tissue and optionally removing fat and epidermal tissue;

(c) mincing the sterilized isolated muscle tissue and placing the minced sections into cell culture media;

(d) incubating the media and minced sections of step (c) at 28^{o}C without CO_2 for 3 to 7 days;

(e) removing the remaining minced sections, adding fresh media, and incubating the remaining cells in the media at 28^{o}C without CO_2 for 3 to 7 days; and

(f) repeating step (e) when cells number about 8 million in 10 ml of culture media, wherein step (f) is repeated at least 10 times.

14. A cell culture comprising the renewable crustacean muscle cell line according to claim 1.

15. A cell culture comprising the renewable crustacean muscle cell line of claim 3.

16. A cell culture comprising the renewable crustacean muscle cell line according to claim 4.

17. A cell culture comprising the renewable crustacean muscle cell line according to claim 7.

18. A cell culture comprising the renewable crustacean muscle cell line according to claim 1, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus*.

19. A cell culture comprising the renewable crustacean muscle cell line according to claim 1, wherein the renewable crustacean muscle cell line is shrimp, and/or prawn muscle cells shrimp, and/or prawn muscle cells are from *Penaeus monodon* and/or *Litopenaeus vannamei*.