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 crustacean muscle stem cell line obtained from isolated primary muscle stem cells, wherein the isolated primary muscle stem cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.
[009b] In another aspect, the present disclosure refers to a crustacean tissue comprising

muscle cells differentiated from the crustacean muscle stem cell line as described herein.

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

[009d] In another aspect, the present disclosure refers to a method for producing a shrimp and/or prawn muscle stem cell line according to claim 1 comprising (a) isolating shrimp and/or prawn muscle; (b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle; (c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise cells; (d) incubating the cell culture of step (c) at 28^{o}C without CO_2 for 3 to 7 days; (e) removing the minced muscle tissue sections from the cell culture, wherein the cells comprised in the muscle tissue sections remain in the cell culture; (f) adding fresh media to the cell culture and incubating the cells in the cell culture at 28^{o}C without CO_2 for 3 to 7 days; and (g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

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

[0009f] In another aspect, the present disclosure refers to a cell culture comprising the crustacean muscle stem cell line as described herein, wherein the crustacean muscle stem 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 crustacean muscle stem cell line as described herein, wherein the crustacean muscle stem cell line is shrimp, and/or prawn muscle stem cells, wherein the shrimp, and/or prawn muscle stem cells are from *Penaeus monodon* and/or *Litopenaeus vannamei*.

[009h] In another aspect, the present disclosure refers to a method for producing a crab muscle stem cell line according to claim 1 comprising (a) isolating crab muscle from claws, legs, and

bodies of freshly sacrificed crabs; (b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle; (c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells; (d) incubating the cell culture of step (c) at 20-24^{o}C without CO_2 for about 7 days; (e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture; (f) adding fresh media to the cell culture and incubating the cells in the cell culture at 20-24^{o}C without CO_2; and (g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

[009i] In another aspect, the present disclosure refers to a method for producing a crayfish or lobster muscle stem cell line according to claim 1 comprising (a) isolating crayfish or lobster muscle from claws, legs, and tails of freshly sacrificed crayfish or lobster; (b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle; (c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells; (d) incubating the cell culture of step (c) at 27^{o}C without CO_2 for about 7 days for crayfish or at saturated humidity at 5^{o}C for lobster; (e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture; (f) adding fresh media to the cell culture and incubating the cells in the cell culture at 27^{o}C without CO_2 for crayfish or at saturated humidity at 5^{o}C for lobster; and (g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

[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

CLAIMS

We claim:

1. A crustacean muscle stem cell line obtained from isolated primary muscle stem cells, wherein the isolated primary muscle stem cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.

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

3. The crustacean muscle stem cell line according to claim 1, wherein the muscle stem cells differentiate into myofibers.

4. The crustacean muscle stem cell line of claim 1, wherein the isolated primary muscle stem cells are shrimp or lobster primary muscle stem cells.

5. A crustacean tissue comprising muscle cells differentiated from the crustacean muscle stem cell line of claim 1.

6. A meat product comprising muscle cells differentiated from the crustacean muscle stem cell line of claim 1.

7. The meat product according to claim 6, further comprising other shrimp, prawn, crab, crayfish, and/or lobster cells.

8. The meat product according to claim 6, wherein the crustacean muscle stem 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.*

9. The meat product according to claim 6, wherein the crustacean muscle stem cell line is

shrimp and/or prawn muscle cells from *Penaeus monodon* and/or *Litopenaeus vannamei*.

10. A method for producing a shrimp and/or prawn muscle stem cell line according to claim 1 comprising

(a) isolating shrimp and/or prawn muscle;

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

(c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise cells;

(d) incubating the cell culture of step (c) at 28°C without CO_2 for 3 to 7 days;

(e) removing the minced muscle tissue sections from the cell culture, wherein the cells comprised in the muscle tissue sections remain in the cell culture;

(f) adding fresh media to the cell culture and incubating the cells in the cell culture at 28°C without CO_2 for 3 to 7 days; and

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

11. A cell culture comprising the crustacean muscle stem cell line according to claim 1.

12. A cell culture comprising the crustacean muscle stem cell line of claim 3.

13. A cell culture comprising the crustacean muscle stem cell line according to claim 4.

14. A method for producing a crab muscle stem cell line according to claim 1 comprising

(a) isolating crab muscle from claws, legs, and bodies of freshly sacrificed crabs;

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

(c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells;

(d) incubating the cell culture of step (c) at 20-24°C without CO_2 for about 7 days;

(e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture;

(f) adding fresh media to the cell culture and incubating the cells in the cell culture at 20-24°C without CO_2; and

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

15. A method for producing a crayfish or lobster muscle stem cell line according to claim 1 comprising

(a) isolating crayfish or lobster muscle from claws, legs, and tails of freshly sacrificed crayfish or lobster;

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

(c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells;

(d) incubating the cell culture of step (c) at 27°C without CO_2 for about 7 days for crayfish or at saturated humidity at 5°C for lobster;

(e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture;

(f) adding fresh media to the cell culture and incubating the cells in the cell culture at 27°C without CO_2 for crayfish or at saturated humidity at 5°C for lobster; and

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

16. A cell culture comprising the crustacean muscle stem cell line according to claim 1, wherein the crustacean muscle stem 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*.

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