From:	amy.sloan@noaa.gov
Subject:	Invitation: Lolita (aka Toki) site visit @ Wed Mar 2, 2022 2pm - 2:30pm (EST)
-	(ruth.ewing@noaa.gov)
To:	ruth.ewing@noaa.gov; Jenny Litz
Sent:	March 1, 2022 6:06 PM (UTC-05:00)
Attached:	invite.ics

Discuss potential for site visit next week at Miami Seaquarium to see Toki

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# Lolita (aka Toki) site visit

# 20220302T190000Z CONFIRMED

#### PRODID

-//Google Inc//Google Calendar 70.9054//EN

#### <u>Version</u>

2.0

### CALSCALE

GREGORIAN

#### **METHOD**

REQUEST

#### Start Date/Time

20220302T190000Z

#### End Date/Time 20220302T193000Z

#### **DTSTAMP**

20220301T230538Z

#### <u>ORGANIZER</u> ( CN=amy.sloan@noaa.gov ) mailto:amy.sloan@noaa.gov

#### UID

1tvjhce7cj1b8o3tqf9etqe7e5@google.com

Attendee mailto:jenny.litz@noaa.gov *Role* REQ-PARTICIPANT *RSVP* TRUE

Attendee mailto:amy.sloan@noaa.gov Role REQ-PARTICIPANT RSVP TRUE

Attendee mailto:ruth.ewing@noaa.gov *Role* REQ-PARTICIPANT *RSVP* TRUE

# X-GOOGLE-CONFERENCE

#### X-MICROSOFT-CDO-OWNERAPPTID -1585046222

#### **Categories**

PRIVATE

#### **CREATED**

20220301T230537Z

#### **Description**

Discuss potential for site visit next week at Miami Seaquarium to see Toki

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#### Last Modified

20220301T230538Z

#### Location

# Sequence Number

0

#### <u>Status</u>

CONFIRMED

### **Summary**

Lolita (aka Toki) site visit

#### **Time Transparency**

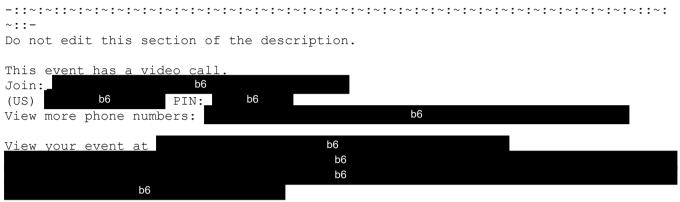
OPAQUE

From:	Ruth Ewing <	b6 >
Subject:	Instagram post	
To:	ruth.ewing@noaa.gov	
Sent:	March 17, 2022 11:28 I	PM (UTC-04:00)
Attached:	Screenshot_20220317-	232644_Instagram.jpg

Commissioner Regalado

From:Ruth Ewing < b6</th>Subject:InstagramTo:ruth.ewing@noaa.govSent:March 17, 2022 11:32 PM (UTC-04:00)Attached:Screenshot\_20220317-233030\_Instagram.jpg

From:	amy.sloan@noaa.gov
Subject:	Invitation: Tentative hold Toki Meeting @ Tue May 31, 2022 4pm - 5pm (EDT)
	(ruth.ewing@noaa.gov)
To:	ruth.ewing@noaa.gov; Deborah Fauquier - NOAA Federal; courtney.smith@noaa.gov
Sent:	May 27, 2022 12:24 PM (UTC-04:00)
Attached:	invite.ics



# **Tentative hold Toki Meeting**

# 20220531T200000Z CONFIRMED

#### PRODID

-//Google Inc//Google Calendar 70.9054//EN

#### <u>Version</u>

2.0

# CALSCALE

GREGORIAN

#### **METHOD**

REQUEST

#### Start Date/Time

20220531T200000Z

#### End Date/Time 20220531T210000Z

#### DTSTAMP

20220527T162357Z

#### <u>ORGANIZER</u> ( CN=amy.sloan@noaa.gov ) mailto:amy.sloan@noaa.gov

#### UID

3nilfbmijil4a55pc7hkd6gnkq@google.com

Attendee		mailto:deborah.fauguier@noaa.gov
Ro	ble	REQ-PARTICIPANT
RSI	IΡ	TRUE

Attendee mailto:amy.sloan@noaa.gov Role REQ-PARTICIPANT RSVP TRUE

Attendee		mailto:ruth.ewing@noaa.gov
	Role	REQ-PARTICIPANT
	RSVP	TRUE

Attendee mailto:courtney.smith@noaa.gov Role REQ-PARTICIPANT RSVP TRUE

X-GOOGLE-CONFERENCE

#### X-MICROSOFT-CDO-OWNERAPPTID 901658251

#### **Categories**

PRIVATE

## CREATED

20220527T162355Z

#### **Description**

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20220527T162355Z

### **Location**

# Sequence Number

0

### <u>Status</u>

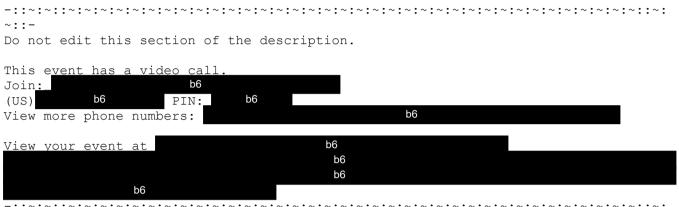
CONFIRMED

#### <u>Summary</u>

Tentative hold Toki Meeting

# Time Transparency OPAQUE

From:amy.sloan@noaa.govSubject:Invitation: Tentative hold Toki Meeting @ Tue May 31, 2022 12:30pm - 1:30pm (EDT)<br/>(ruth.ewing@noaa.gov)To:ruth.ewing@noaa.gov; deborah.fauquier@noaa.gov; courtney.smith@noaa.govSent:May 27, 2022 12:24 PM (UTC-04:00)Attached:invite.ics



# **Tentative hold Toki Meeting**

# 20220531T163000Z CONFIRMED

#### PRODID

-//Google Inc//Google Calendar 70.9054//EN

#### <u>Version</u>

2.0

# CALSCALE

GREGORIAN

#### **METHOD**

REQUEST

#### Start Date/Time

20220531T163000Z

#### End Date/Time 20220531T173000Z

#### **DTSTAMP**

20220527T162414Z

#### <u>ORGANIZER</u> ( CN=amy.sloan@noaa.gov ) mailto:amy.sloan@noaa.gov

#### UID

7vedlbgmdu7a6eaggu5kkhsqip@google.com

- Attendee mailto:ruth.ewing@noaa.gov Role REQ-PARTICIPANT RSVP TRUE
- Attendee mailto:amy.sloan@noaa.gov Role REQ-PARTICIPANT RSVP TRUE
- Attendee mailto:deborah.fauquier@noaa.gov *Role* REQ-PARTICIPANT *RSVP* TRUE
- Attendee mailto:courtney.smith@noaa.gov Role REQ-PARTICIPANT RSVP TRUE

X-GOOGLE-CONFERENCE

#### X-MICROSOFT-CDO-OWNERAPPTID 176210996

#### **Categories**

PRIVATE

## CREATED

20220527T162251Z

#### **Description**

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#### Last Modified

20220527T162412Z

## **Location**

# Sequence Number

0

### <u>Status</u>

CONFIRMED

#### <u>Summary</u>

Tentative hold Toki Meeting

# Time Transparency OPAQUE

From:	Ruth Ewing - NOAA Federal <ruth.ewing@noaa.gov></ruth.ewing@noaa.gov>
Subject:	Re: Lolita samples and measurements for NWFSC and SWFSC
To:	phillip.morin@noaa.gov
Cc:	Amy Sloan - NOAA Federal; Marlys Houck
Sent:	October 4, 2022 1:51 PM (UTC-04:00)

Hi Phil,

With respect to your question,

"Do you know if necropsy is likely to be delayed more than a few hours due to the end-of life events, and whether the tissue samples for cell culture can be taken in time to preserve the live cells (i.e., a few days for skin samples if kept cool, but not frozen; less for internal tissues)?"

It is quite possible that sampling could be delayed especially if she dies during the night. I will discuss with Seaquarium's veterinarian the possibility of obtaining skin samples prior to Toki being transported to Georgia for the necropsy. That way the skin samples at the very least can be harvested and stored in a refrigerator in Miami without the worry of additional degradation as time passes during transport.

This does include the samples for the Frozen Zoo cell culture. Sampling of the internal organs - is there a point in time at which those samples are not suitable for cell culture but are still desired for other genomic work?

Ruth

PS - If you and Marly are available would either of you be able to present to the SEFSC Marine Mammal and Turtle Division about the Frozen Zoo cell culture work and the broader need for samples from our region. Thanks.

On Tue, Oct 4, 2022 at 12:50 PM Phil Morin (NOAA Federal) cphillip.morin@noaa.gov wrote:

Hi Ruth,

I received the email from Amy Sloan (cc'd) today about the declining health of Lolita. That's sad news, but I'm glad that you have the biopsy media on hand now in the event that Lolita dies or has to be euthanized, as well as for other species that can be added to the frozen zoo biobank.

The email referred to an "eventual necropsy" at the University of Georgia and collection of samples for NWFSC and SWFSC, which I assume includes cell culture at the San Diego Wildlife Alliance' Frozen Zoo (Marlys also cc'd). Do you know if necropsy is likely to be delayed more than a few hours due to the end-of life events, and whether the tissue samples for cell culture can be taken in time to preserve the live cells (i.e., a few days for skin samples if kept cool, but not frozen; less for internal tissues)?

If there are any questions on sample collections, handling, or shipping, I am available by email, phone or text.

Sincerely,

Phil Morin (mobile: 858-405-5030)

--Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165 phillip.morin@noaa.gov http://swfsc.noaa.gov/mmtd-mmgenetics

"I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown

#### On 7/25/22 12:34 PM, Marlys Houck wrote:

Hi Ruth,

The FedEx tracking # for the biopsy vials headed your way is: b6

It should arrive Wednesday.

Thanks!

Marlys

From: Marlys Houck
Sent: Thursday, July 21, 2022 1:20 PM
To: Ruth Ewing - NOAA Federal <a href="mailto:ruth.ewing@noaa.gov">ruth.ewing@noaa.gov</a>; Phillip Morin - NOAA Federal <<u>phillip.morin@noaa.gov></u>
Cc: Amy Sloan - NOAA Federal <<u>a my.sloan@noaa.gov></u>
Subject: RE: Lolita samples and measurements for NWFSC and SWFSC

Hi Ruth,

Thanks for your interest in contributing to the Frozen Zoo. I'll get some biopsy vials sent out to you by Weds. next week. I'll send the tracking number when we generate it.

Generally the samples are collected post mortem, and as Phil mentioned, this needs to occur while the tissue is still fresh and contains living cells. In warm climates the tissue will start to decay more quickly than in cooler areas, but as a general rule the samples should be placed into the biopsy vials in 24 hours or less (sooner is better) and shipped that day or next morning if possible.

We are open to receiving other species including sea turtles at sea. We aren't permitted for sea turtles on land yet. We did finally get our manatee permit and froze our first cell line from this species a few weeks ago; so exciting!

I believe Phil has a link to a biopsy collection protocol adapted for NOAA staff and I'm happy to answer any questions you have.

Thanks again,

Marlys

Marlys Houck CG(ASCP)<sup>CM</sup>

Curator of the Frozen Zoo®, Reproductive Sciences

**Conservation Science** 



15600 San Pasqual Valley Road

Escondido, CA 92027

760-291-5454

mhouck@sdzwa.org

From: Ruth Ewing - NOAA Federal <<u>ruth.ewing@noaa.gov</u>>
Sent: Thursday, July 21, 2022 10:34 AM
To: Phillip Morin - NOAA Federal <<u>phillip.morin@noaa.gov</u>>
Cc: Amy Sloan - NOAA Federal <<u>amy.sloan@noaa.gov</u>>; Marlys Houck <<u>MHouck@sdzwa.org</u>>
Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Hello Maryls,

Thank you for providing the material needed. I believe I saw your IAAAM presentation recently regarding the frozen zoo. I believe you were also looking to include samples from other species as well including sea turtles?

If we are lucky enough to continue to have our SRKW around for a while I would like to maybe send you material from other animals to enhance your collection.

My address is:

NOAA Fisheries

Attn: Ruth Ewing

75 Virginia Beach Dr

Miami FL 33149

my cell number is 305-505-0895

Regards,

Ruth

On Thu, Jul 21, 2022 at 1:17 PM Phil Morin (NOAA Federal) phillip.morin@noaa.gov wrote:

Hi Ruth,

I checked with Marlys (cc'd), and she is happy to send you the tissue storage media vials and protocol next week. All she needs is the shipping address and phone number from you.

Once you have these on hand, they can be stored in the refrigerator for up to a year typically, so they will be available without delay if needed.

Cheers,

Phil

On 7/20/22 5:11 PM, Phil Morin (NOAA Federal) wrote:

Hi Ruth, and thanks for the quick reply.

I am not aware of any SRKW samples that could be used for cell lines. They have to be collected from living tissues (which can survive after animal death, but only for a matter of hours to days without appropriate preservation). The preservation method is specific to live cells, so we can't use frozen tissues or tissues put in ethanol or other preservatives.

I will ask Marlys to send you some vials to have on hand. She does that regularly, so I'm sure she'll be happy to do it, and include the protocols for sampling, storage and shipping if samples are eventually collected.

Cheers,

Phil

On 7/20/22 4:35 PM, Ruth Ewing - NOAA Federal wrote:

Hello Phillip,

Indeed, Lolita has recovered well but is still being watched closely. The sampling protocols are in place ; however, I'm not certain the cell culture vials from Marlys were received. Are there any tissues that could be used now to attempt to establish a cell line from a SRKW?

Regards,

Ruth

On Wed, Jul 20, 2022 at 6:41 PM Phil Morin (NOAA Federal) <<u>phillip.morin@noaa.gov</u>> wrote:

Hi Ruth,

I was in a meeting today discussing killer whale toxicology and contaminant loads, and some of participants have established a cell line from a transient killer whale from the Puget Sound area, and how they can use that for their contaminant effects study. It reminded me that we were hoping to start a cell line (and get other tissues) from Lolita in the event of her death.

I assume Lolita recovered from her health scare in February, and hope she's doing well. We would still like to make sure sampling protocols and supplies are in place if/when needed, and I'm not sure what ended up happening back in February. Did you receive cell culture vials from Marlys at the San Diego Zoo Wildlife Alliance?

Thanks,

Phil ---Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165 phillip.morin@noaa.gov http://swfsc.noaa.gov/mmtd-mmgenetics

"I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown

#### On 2/11/22 12:02 PM, Marlys Houck wrote:

Hi Ruth,

The vials can be stored in the refrigerator for at least a year, and can tolerate several days at room temp if needed. They are small glass vials with pink, clear fluid. As long as the media remains pink and clear they are good. Yellow and/or cloudy media would indicate contamination and those should be discarded, however, that's a very rare occurrence.

It looks like you were going to ask another question (yellow highlight below)?

Best,

Marlys

From: Ruth Ewing - NOAA Federal <<u>ruth.ewing@noaa.gov></u>
Sent: Friday, February 11, 2022 11:14 AM
To: Marlys Houck <<u>MHouck@sdzwa.org></u>
Cc: Phillip Morin - NOAA Federal <<u>phillip.morin@noaa.gov></u>
Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Hello Marlys,

I read through the protocol, my question is regarding the biopsy transport vials to protect the cell culture tissues during shipment. Is there an expiration date on these vials or can they be stored in an -80C to prolong the shelf life of the media?

Thanks,

Ruth

Also are you

On Fri, Feb 11, 2022 at 1:44 PM Marlys Houck <<u>MHouck@sdzwa.org</u>> wrote:

Dear Ruth,

Thank you for considering taking these important samples. As Phil mentioned, I can provide biopsy transport vials to protect the cell culture tissues culture shipment. The vials can be stored in the refrigerator for at least a year, and can tolerate several days at room temp if needed. All I need is your shipping address and an associated phone number (required by FedEx). We generally ship on a Monday for arrival on Wednesday.

Thanks again,

Marlys

Marlys Houck CG(ASCP)<sup>CM</sup>

Curator of the Frozen Zoo®, Reproductive Sciences

**Conservation Science** 



15600 San Pasqual Valley Road

Escondido, CA 92027

760-291-5454

mhouck@sdzwa.org

From: Phil Morin (NOAA Federal) <<u>phillip.morin@noaa.gov</u>> Sent: Friday, February 11, 2022 10:10 AM To: Dawn Noren - NOAA Federal <<u>dawn.noren@noaa.gov</u>>; Ruth Ewing -NOAA Federal <<u>ruth.ewing@noaa.gov</u>> Cc: Kim Parsons - NOAA Federal <<u>kim.parsons@noaa.gov</u>>; Linda Rhodes -NOAA Federal <<u>linda.rhodes@noaa.gov</u>>; Irvin Schultz - NOAA Federal <<u>irvin.schultz@noaa.gov</u>>; Kathi Lefebvre - NOAA Federal <<u>kathi.lefebvre@noaa.gov</u>>; Brad Hanson - NOAA Federal <<u>brad.hanson@noaa.gov</u>>; Mike Ford - NOAA Federal <<u>Mike.Ford@noaa.gov</u>>; Marlys Houck <<u>MHouck@sdzwa.org</u>> Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Dear Ruth,

I'm responding from the SWFSC. We are very interested in obtaining a variety of samples that could be useful for ongoing and future research, including establishment of cell lines archived in the "Frozen Zoo" cell culture collection a the San Diego Zoo Wildlife Alliance. We have been working with the Frozen Zoo for over 15 years, and have established cell lines for 17 cetacean species to date, some of which have been used to generate the reference genomes for the species.

I've cc'd Marlys Houck, the curator of the Frozen Zoo collection, so that she can communicate with you directly and send the tissue culture media vials needed to preserve and protect the cells prior to shipping to San Diego. We also have a protocol for sampling on the <u>Cetacean Genomes</u> <u>Project web site</u>. Multiple tissues would be optimal to get the best chance of growing cells, but even just skin is often sufficient.

In addition to establishing a cell line, we are interested in multiple tissues for genetics/genomics work, included in the spreadsheet sent by Dawn from the NWFSC. For the SWFSC samples, we have included both frozen samples (-80) of multiple tissues, and tissues in RNAlater. If you don't have RNAlater storage solution, I will see if we can send some to you.

While I hope Lolita regains her health, I also hope we can be prepared to advance killer whale studies with the valuable samples and data you can provide.

Sincerely,

Phil Morin

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--

Phillip A. Morin, Ph.D.

(he/him/his)

Southwest Fisheries Science Center

8901 La Jolla Shores Dr.

La Jolla, CA 92037, USA

Phone: 858-546-7165

phillip.morin@noaa.gov

http://swfsc.noaa.gov/mmtd-mmgenetics
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"No one worked on all of those successes. But someone worked on each of them, and that's what made the difference." - Carl Safina (Psychic Numbing: Keeping Hope Alive in a World of Extinctions).

#### On 2/11/22 9:53 AM, Dawn Noren - NOAA Federal wrote:

Dear Ruth-

Thanks very much for your assistance. I have collated and organized the NWFSC and SWFSC requests. Some of the samples require specific collection containers, which we have noted. For example, serum tubes for chemistry should not have the gel separators. Please let us know if we should send down supplies. Each PI, copied on this email, can be contacted for further details. I have attached four documents.

1) Summary of measurements and samples requested divided by code 1 and necropsy. A diagram showing a particular sample site for the longissiumus dorsi is also included.

2) PDF protocol and data sheet for body morphometric measurements. Page 1 is the highest priority (3 of the 6 blubber thickness measurements are also prioritized) and page 2 is the sheet from the killer whale necropsy protocol. As indicated on the document, page 1 covers all of the priority body measurements from the necropsy protocol. There are three additional appendage measurements on page 2 that are also important.

3) Excel sheet for necropsy samples only organized by sample type and investigator. I grouped samples in what I thought was a logical order during a necropsy, but feel free to re-sort as needed.

4) Killer whale necropsy protocol. I know you have it already, but I just wanted to make sure it was grouped with the other documents in the email. The summary document refers to specific pages in the necropsy protocol.

Please let us know if you have any questions or need more information.

Thanks,

Dawn

Dawn P. Noren, Ph.D. Research Fishery Biologist NOAA NMFS Northwest Fisheries Science Center 2725 Montlake Blvd. East Seattle, WA 98112 (206) 302-2439 https://www.fisheries.noaa.gov/contact/dawn-p-norenadams-phd \_\_\_ Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165 phillip.morin@noaa.gov http://swfsc.noaa.gov/mmtd-mmgenetics "I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown \_\_\_ \_\_\_ Phillip A. Morin, Ph.D. (he/him/his)

Southwest Fisheries Science Center

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Phone: 858-546-7165

phillip.morin@noaa.gov

http://swfsc.noaa.gov/mmtd-mmgenetics

"I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown

From:	Ruth Ewing - NOAA Federal <ruth.ewing@noaa.gov></ruth.ewing@noaa.gov>
Subject:	Re: Lolita samples and measurements for NWFSC and SWFSC
To:	Marlys Houck
Cc:	phillio.morin@noaa.gov: Amy Sloan - NOAA Federal
Cc:	phillip.morin@noaa.gov; Amy Sloan - NOAA Federal
Sent:	October 4, 2022 5:46 PM (UTC-04:00)

Hi Marlys,

Gonads on whales are internal, so that won't work, however, the sublingual gingiva (frenulum) could work.

Could a site prep be done with alcohol to reduce contamination and then use a sterile water or sterile saline rinse prior to sampling? Just a thought.

As for the punch size - I only have 6mm biopsy punches.

As for the seminar speaking engagement- The 26th is perfect. And I think the IAAAM ppt is perfect for the audience. I am thrilled to have either or both of you present as a tag team and best suited to your schedules. Our Turtle folks did ask me to invite Peter Dutton from the SWFSC Sea Turtle Genetics Lab as well.

I'll send out a placeholder google invite for you two to double check you calendars... Would you be able to provide a brief abstract and the title? The one from IAAAM would be fine for me to include in the invite. The seminars are not recorded and are conducted through Google Meet.

Thank you so much, Ruth

On Tue, Oct 4, 2022 at 3:24 PM Marlys Houck <<u>MHouck@sdzwa.org</u>> wrote:

Hi Ruth and Phil,

Thanks for all the careful planning. Another site for external tissues would be gonads on males (assuming they are external?). We haven't tried many samples from inside the mouth. I used to try it on rhinos back in the day without much success, but am definitely open to trying it on such a different species. We have good luck with amphibian tongues, so that could be an option. Probably a 6mm deep and 8+mm surface diameter section. Another good option is to take 2-3 skin biopsies (~8mm diameter and 6mm deep) and place each in separate biopsy vials. Sometimes one area has contamination and another doesn't.

Ruth, sorry to be late answering the earlier invitation you sent to me about speaking at your meeting on a Wednesday. I'm available only 10/26 as far as Wednesdays go this month. I'm also happy to have Phil do the presentation (thanks for the offer Phil!) since I'm a bit under water right now. As Phil said, he knows the topic very well. Phil, I can send you the ppt I recently did for International Association for Aquatic Animal Medicine that was geared towards field collection of marine mammals and sea turtles to use what you like.

Marlys

From: Phil Morin (NOAA Federal) <<u>phillip.morin@noaa.gov</u>>
Sent: Tuesday, October 4, 2022 11:16 AM
To: Ruth Ewing - NOAA Federal <<u>ruth.ewing@noaa.gov</u>>
Cc: Amy Sloan - NOAA Federal <<u>amy.sloan@noaa.gov</u>>; Marlys Houck <<u>MHouck@sdzwa.org</u>>
Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Hi Ruth,

Collection of skin samples prior to transfer to Georgia would be the best, both for rapid freezing (for DNA and other biomaterials that can be preserved at -80), and for cell culture (in the cell culture media, stored at  $\geq 4^{\circ}$ C and shipped ASAP to the Frozen Zoo).

Internal tissues may still be viable after a few days, but it's much less likely and highly dependent on how quickly they cool down, and that

they do not freeze. In this case, getting the fresh the skin samples gives us the best chance, and collecting additional tissues should be discussed after we know better what the time lag and storage conditions will be. Marlys may have ideas for getting additional "external" tissue samples that could be of use (e.g., inside the mouth?).

For other genomic work, some internal organ sampling will still be worthwhile. RNA will degrade quickly, so the tissues are not likely to be of much use for RNA if they are not harvested quickly (within a day or so, possibly longer if there is cold storage until the necropsy). DNA and many other biomolecules are more robust, so it's still worth while to collect the tissues for cryopreservation as soon as possible (-80°C). Documentation of the time delay and conditions from death to harvesting will help us to know what the tissue will be most useful for.

When the time comes, it will probably be best to talk on the phone to assess the situation and make decisions.

Regarding giving a presentation, I would be happy to do one for you, and can either do it together with Marlys or, if she's unavailable, I can get some materials from her to present about the Frozen Zoo collection. I've known Marlys for 35 years and have worked with her on the cetacean collections for >15 years, so I have a reasonable understanding of the logistics and potential uses of the samples in the FZ collection (at least for marine mammals).

Cheers,

Phil

On 10/4/22 10:50 AM, Ruth Ewing - NOAA Federal wrote:

Hi Phil,

With respect to your question,

"Do you know if necropsy is likely to be delayed more than a few hours due to the end-of life events, and whether the tissue samples for cell culture can be taken in time to preserve the live cells (i.e., a few days for skin samples if kept cool, but not frozen; less for internal tissues)?"

It is quite possible that sampling could be delayed especially if she dies during the night. I will discuss with Seaquarium's veterinarian the possibility of obtaining skin samples prior to Toki being transported to Georgia for the necropsy. That way the skin samples at the very least can be harvested and stored in a refrigerator in Miami without the worry of additional degradation as time passes during transport.

This does include the samples for the Frozen Zoo cell culture. Sampling of the internal organs - is there a point in time at which those samples are not suitable for cell culture but are still desired for other genomic work?

Ruth

PS - If you and Marly are available would either of you be able to present to the SEFSC Marine Mammal and Turtle Division about the Frozen Zoo cell culture work and the broader need for samples from our region. Thanks.

On Tue, Oct 4, 2022 at 12:50 PM Phil Morin (NOAA Federal) phillip.morin@noaa.gov> wrote:

Hi Ruth,

I received the email from Amy Sloan (cc'd) today about the declining health of Lolita. That's sad news, but I'm glad that you have the biopsy media on hand now in the event that Lolita dies or has to be euthanized, as well as for other species that can be added to the frozen zoo biobank.

The email referred to an "eventual necropsy" at the University of Georgia and collection of samples for NWFSC and SWFSC, which I assume includes cell culture at the San Diego Wildlife Alliance' Frozen Zoo (Marlys also cc'd). Do you know if necropsy is likely to be delayed more than a few hours due to the end-of life events, and whether the tissue samples for cell culture can be taken in time to preserve the live cells (i.e., a few days for skin samples if kept cool, but not frozen;

less for internal tissues)? If there are any questions on sample collections, handling, or shipping, I am available by email, phone or text. Sincerely, Phil Morin (mobile: 858-405-5030) \_\_\_ \_\_\_ Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165 phillip.morin@noaa.gov http://swfsc.noaa.gov/mmtd-mmgenetics "I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown On 7/25/22 12:34 PM, Marlys Houck wrote: Hi Ruth, The FedEx tracking # for the biopsy vials headed your way is: b6 It should arrive Wednesday. Thanks! Marlys From: Marlys Houck Sent: Thursday, July 21, 2022 1:20 PM

To: Ruth Ewing - NOAA Federal <u><ruth.ewing@noaa.gov></u>; Phillip Morin - NOAA Federal <u><phillip.morin@noaa.gov></u> Cc: Amy Sloan - NOAA Federal <u><amy.sloan@noaa.gov></u> Subject: RE: Lolita samples and measurements for NWFSC and SWFSC

Hi Ruth,

Thanks for your interest in contributing to the Frozen Zoo. I'll get some biopsy vials sent out to you by Weds. next week. I'll send the tracking number when we generate it.

Generally the samples are collected post mortem, and as Phil mentioned, this needs to occur while the tissue is still fresh and contains living cells. In warm climates the tissue will start to decay more quickly than in cooler areas, but as a general rule the samples should be placed into the biopsy vials in 24 hours or less (sooner is better) and shipped that day or next morning if possible.

We are open to receiving other species including sea turtles at sea. We aren't permitted for sea turtles on land yet. We did finally get our manatee permit and froze our first cell line from this species a few weeks ago; so exciting!

I believe Phil has a link to a biopsy collection protocol adapted for NOAA staff and I'm happy to answer any questions you have.

Thanks again,

Marlys

Marlys Houck CG(ASCP)<sup>CM</sup>

Curator of the Frozen Zoo®, Reproductive Sciences

**Conservation Science** 



15600 San Pasqual Valley Road

Escondido, CA 92027

760-291-5454

mhouck@sdzwa.org

From: Ruth Ewing - NOAA Federal <<u>ruth.ewing@noaa.gov</u>>
Sent: Thursday, July 21, 2022 10:34 AM
To: Phillip Morin - NOAA Federal <<u>phillip.morin@noaa.gov</u>>
Cc: Amy Sloan - NOAA Federal <<u>amy.sloan@noaa.gov</u>>; Marlys Houck <<u>MHouck@sdzwa.org</u>>
Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Hello Maryls,

Thank you for providing the material needed. I believe I saw your IAAAM presentation recently regarding the frozen zoo. I believe you were also looking to include samples from other species as well including sea turtles?

If we are lucky enough to continue to have our SRKW around for a while I would like to maybe send you material from other animals to enhance your collection.

My address is:

NOAA Fisheries

Attn: Ruth Ewing

75 Virginia Beach Dr

Miami FL 33149

my cell number is 305-505-0895

Regards,

Ruth

On Thu, Jul 21, 2022 at 1:17 PM Phil Morin (NOAA Federal) phillip.morin@noaa.gov wrote:

Hi Ruth,

I checked with Marlys (cc'd), and she is happy to send you the tissue storage media vials and protocol next week. All she needs is the shipping address and phone number from you.

Once you have these on hand, they can be stored in the refrigerator for up to a year typically, so they will be available without delay if needed.

Cheers,

Phil

On 7/20/22 5:11 PM, Phil Morin (NOAA Federal) wrote:

Hi Ruth, and thanks for the quick reply.

I am not aware of any SRKW samples that could be used for cell lines. They have to be collected from living tissues (which can survive after animal death, but only for a matter of hours to days without appropriate preservation). The preservation method is specific to live cells, so we can't use frozen tissues or tissues put in ethanol or other preservatives.

I will ask Marlys to send you some vials to have on hand. She does that regularly, so I'm sure she'll be happy to do it, and include the protocols for sampling, storage and shipping if samples are eventually collected.

Cheers,

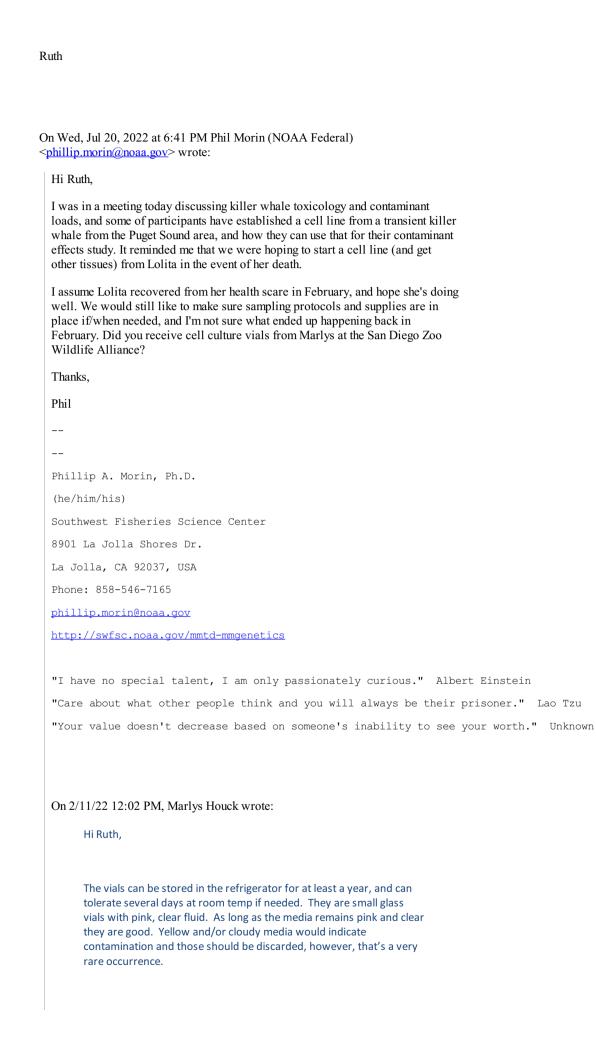
Phil

On 7/20/22 4:35 PM, Ruth Ewing - NOAA Federal wrote:

Hello Phillip,

Indeed, Lolita has recovered well but is still being watched closely. The sampling protocols are in place ; however, I'm not certain the cell culture vials from Marlys were received. Are there any tissues that could be used now to attempt to establish a cell line from a SRKW?

Regards,



It looks like you were going to ask another question (yellow highlight below)?

Best.

Marlys

From: Ruth Ewing - NOAA Federal <<u>ruth.ewing@noaa.gov></u>
Sent: Friday, February 11, 2022 11:14 AM
To: Marlys Houck <<u>MHouck@sdzwa.org></u>
Cc: Phillip Morin - NOAA Federal <<u>phillip.morin@noaa.gov></u>
Subject: Re: Lolita samples and measurements for NWFSC and SWFSC

Hello Marlys,

I read through the protocol, my question is regarding the biopsy transport vials to protect the cell culture tissues during shipment. Is there an expiration date on these vials or can they be stored in an - 80C to prolong the shelf life of the media?

Thanks,

Ruth

#### Also are you

On Fri, Feb 11, 2022 at 1:44 PM Marlys Houck <<u>MHouck@sdzwa.org</u>> wrote:

Dear Ruth,

Thank you for considering taking these important samples. As Phil mentioned, I can provide biopsy transport vials to protect the cell culture tissues culture shipment. The vials can be stored in the refrigerator for at least a year, and can tolerate several days at room temp if needed. All I need is your shipping address and an associated phone number (required by FedEx). We generally ship on a Monday for arrival on Wednesday.

Thanks again,

Marlys

Marlys Houck CG(ASCP)CM

Curator of the Frozen Zoo®, Reproductive Sciences

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From: Phil Morin (NOAA Federal) <phillip.morin@noaa.gov>
Sent: Friday, February 11, 2022 10:10 AM
To: Dawn Noren - NOAA Federal <dawn.noren@noaa.gov>; Ruth
Ewing - NOAA Federal <ruth.ewing@noaa.gov>
Cc: Kim Parsons - NOAA Federal <kim.parsons@noaa.gov>; Linda
Rhodes - NOAA Federal linda.rhodes@noaa.gov>; Irvin Schultz NOAA Federal linda.rhodes@noaa.gov>; Kathi Lefebvre - NOAA
Federal <kathi.lefebvre@noaa.gov>; Brad Hanson - NOAA Federal
<br/>brad.hanson@noaa.gov>; Marlys Houck <MHouck@sdzwa.org>
Subject: Re: Lolita samples and measurements for NWFSC and

Dear Ruth,

I'm responding from the SWFSC. We are very interested in obtaining a variety of samples that could be useful for ongoing and future research, including establishment of cell lines archived in the "Frozen Zoo" cell culture collection a the San Diego Zoo Wildlife Alliance. We have been working with the Frozen Zoo for over 15 years, and have established cell lines for 17 cetacean species to date, some of which have been used to generate the reference genomes for the species.

I've cc'd Marlys Houck, the curator of the Frozen Zoo collection, so that she can communicate with you directly and send the tissue culture media vials needed to preserve and protect the cells prior to shipping to San Diego. We also have a protocol for sampling on the <u>Cetacean Genomes Project web site</u>. Multiple tissues would be optimal to get the best chance of growing cells, but even just skin is often sufficient.

In addition to establishing a cell line, we are interested in multiple tissues for genetics/genomics work, included in the spreadsheet sent by Dawn from the NWFSC. For the SWFSC samples, we have included both frozen samples (-80) of multiple tissues, and tissues in RNAlater. If you don't have RNAlater storage solution, I will see if we can send some to you.

While I hope Lolita regains her health, I also hope we can be prepared to advance killer whale studies with the valuable samples and data you can provide.

Sincerely,

Phil Morin

--Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165 phillip.morin@noaa.gov http://swfsc.noaa.gov/mmtd-mmgenetics

"No one worked on all of those successes. But someone worked on each of them, and that's what made the difference." - Carl Safina (Psychic Numbing: Keeping Hope Alive in a World of Extinctions).

#### On 2/11/22 9:53 AM, Dawn Noren - NOAA Federal wrote:

#### Dear Ruth-

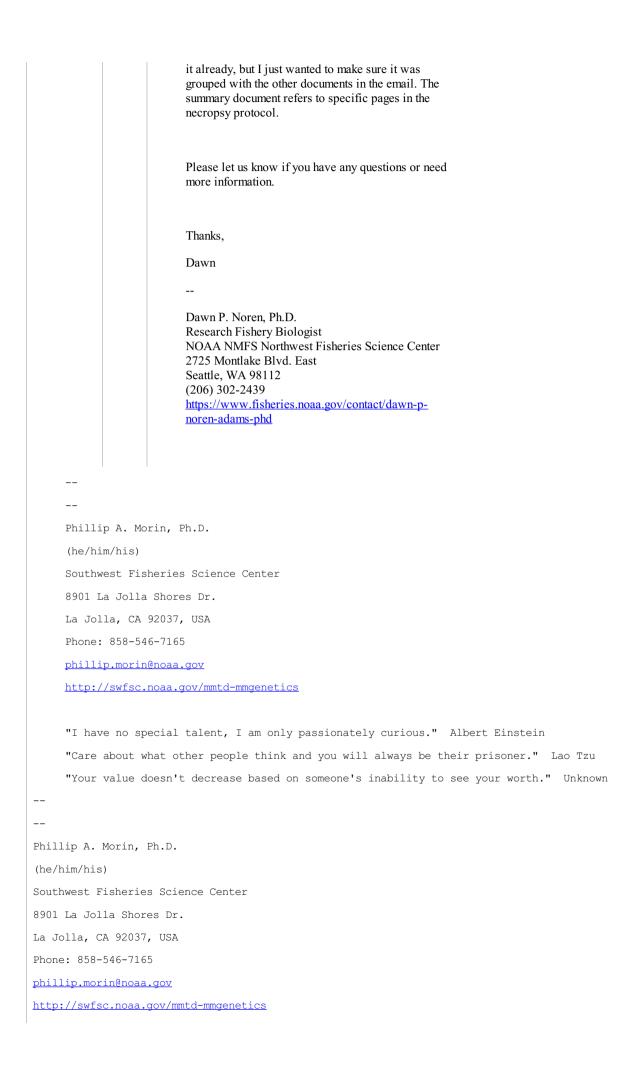
Thanks very much for your assistance. I have collated and organized the NWFSC and SWFSC requests. Some of the samples require specific collection containers, which we have noted. For example, serum tubes for chemistry should not have the gel separators. Please let us know if we should send down supplies. Each PI, copied on this email, can be contacted for further details. I have attached four documents.

1) Summary of measurements and samples requested divided by code 1 and necropsy. A diagram showing a particular sample site for the longissiumus dorsi is also included.

2) PDF protocol and data sheet for body morphometric measurements. Page 1 is the highest priority (3 of the 6 blubber thickness measurements are also prioritized) and page 2 is the sheet from the killer whale necropsy protocol. As indicated on the document, page 1 covers all of the priority body measurements from the necropsy protocol. There are three additional appendage measurements on page 2 that are also important.

3) Excel sheet for necropsy samples only organized by sample type and investigator. I grouped samples in what I thought was a logical order during a necropsy, but feel free to re-sort as needed.

4) Killer whale necropsy protocol. I know you have



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"I have no special talent, I am only passionately curious." Albert Einstein "Care about what other people think and you will always be their prisoner." Lao Tzu "Your value doesn't decrease based on someone's inability to see your worth." Unknown Phillip A. Morin, Ph.D. (he/him/his) Southwest Fisheries Science Center 8901 La Jolla Shores Dr. La Jolla, CA 92037, USA Phone: 858-546-7165

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