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## **MORTALITY INVESTIGATION OF THE MEKONG IRRAWADDY RIVER DOLPHIN (*ORCAELLA BREVIROSTRIS*) IN CAMBODIA BASED ON NECROPSY SAMPLE ANALYSIS**

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## សេចក្តីសង្ខេប

សត្វផ្សោតក្បាលត្រឡោកទន្លេមេគង្គ (*Orcaella brevirostris*) គឺជាសត្វផ្សោតមួយក្រុម ក្នុងចំណោមសត្វផ្សោតទន្លេចំនួនបីក្រុមផ្សេងគ្នា ដែលត្រូវបានចាត់ថ្នាក់ជាប្រភេទរងគ្រោះបំផុតឈាន ទៅរកភាពផុតពូជ។ បច្ចុប្បន្ន ចំនួនប៉ាន់ប្រមាណសរុបនៃសត្វផ្សោតក្បាលត្រឡោកទន្លេមេគង្គគឺស្ថិតនៅ ចន្លោះពី ៦៦ ទៅ ៨៦ក្បាល ប៉ុណ្ណោះ។ ចាប់ពីឆ្នាំ ២០០៣ រហូតមកទល់បច្ចុប្បន្ននេះ សត្វផ្សោតសរុប ចំនួន ៨៨ ក្បាលបានស្លាប់។ អាត្រាស្លាប់ដ៏ខ្ពស់នេះកំពុងធ្វើឱ្យសត្វផ្សោតក្បាលត្រឡោកទន្លេមេគង្គក្លាយ ជាក្រុមសត្វផ្សោតដែលទទួលរងការគំរាមកំហែងខ្លាំងជាងគេក្នុងចំណោមសត្វផ្សោតទន្លេទាំងបីក្រុម និង កំពុងឈានទៅរកភាពវិនាសផុតពូជតែម្តង។

របាយការណ៍នេះ កត់ត្រាលទ្ធផលដែលទទួលបានពីការវិភាគលើសំណាកសត្វផ្សោតស្លាប់តាម រយៈការសិក្សាចំនួនពីរដាច់ដោយឡែកពីគ្នា ដូចខាងក្រោម៖

## ការសិក្សាប្រភេទទី១

ការវិភាគដើម្បីពិនិត្យមើលប្រភេទបាក់តេរីដែលបង្កឱ្យមានជំងឺ ត្រូវបានធ្វើឡើងលើសំណាក ដែលប្រមូលបានពីសត្វផ្សោតដែលស្លាប់ចំនួន ១១ក្បាល ចាប់តាំងពី ឆ្នាំ២០០៧។ ការវិភាគនេះបានធ្វើ ឡើងនៅមន្ទីរពិសោធន៍វិជ្ជាសាស្ត្របារាំងនៃវិទ្យាស្ថានប៉ារីសក្រុងភ្នំពេញនៃប្រទេសកម្ពុជា ។

## ការសិក្សាប្រភេទទី២

សំណាកសត្វផ្សោតចំនួន ២១ដែលប្រមូលបានពីការវះកាត់នៅក្នុងចន្លោះឆ្នាំ ២០០៤ និង២០០៦ ត្រូវ បានសម្រាំងជ្រើសរើស និងផ្ញើរចេញទៅកាន់មន្ទីរពិសោធន៍នៅប្រទេសកាណាដា និងសហរដ្ឋអាមេរិក។ សំណាកទាំងនោះត្រូវបានវិភាគរកប្រភេទរោគសញ្ញាផ្សេងៗ ដូចខាងក្រោម៖

- ពិនិត្យមើលប្រភេទបាក់តេរីដែលបង្កឱ្យសត្វផ្សោតមានជំងឺ ។  
PCR Pathogen (Disease) screening
- ពិនិត្យមើលបំបែបនៃជាលិកាស្បែកដែលបង្កឡើងដោយជំងឺ ។  
Histopathology (microscopy)
- ពិនិត្យមើលជាតិគីមីពុលដែលមាននៅក្នុងបរិស្ថាន ។  
Toxicology (environmental contaminants)
- ពិនិត្យមើលពីសារធាតុលោហៈ ( បារីត និងសេឡេញ៉ូម )  
Heavy Metal (Mercury and Selenium)
- វិភាគមើលជាតិពុលឌីអុកស៊ីន (Dioxin/Furan analysis)
- វិភាគមើលពីទំនាក់ទំនងហ្សែន (Genetic analysis)

**ការសិក្សាប្រភេទទី១**

លទ្ធផលនៃការសិក្សាពីការវិភាគរកមើលប្រភេទបាក់តេរី ដែលបង្កជម្ងឺដល់សត្វផ្សោតបានធ្វើឡើងនៅវិទ្យាស្ថានប៉ាស្ទ័រក្នុងក្រុងភ្នំពេញបានកំណត់ពីមុខសញ្ញានៃប្រភេទជម្ងឺ ដែលជាមូលហេតុនាំឱ្យសត្វផ្សោត ចំនួន១០ក្បាល ស្លាប់នៅក្នុងចំណោមសំណាកសត្វផ្សោតសរុបចំនួន១១ដែលត្រូវបានធ្វើការវិភាគនៅមន្ទីរពិសោធន៍។ ភាគច្រើននៃសាកសពសត្វផ្សោតដែលស្លាប់ប្រមូលបានភ្លាម គឺមានស្នាមនៅត្រង់កមើលទៅដូចជាស្នាមជាំក្រមៅ។ នៅពេលធ្វើការវះកាត់ គេសង្កេតឃើញថាស្នាមជាំនៅត្រង់កទាំងនេះហាក់ដូចជាដំបៅរលួយ។ បាក់តេរីមួយប្រភេទក្នុងចំណោមបាក់តេរីជាច្រើនប្រភេទដែលបង្កឱ្យមានស្នាមជាំនៅត្រង់កនេះត្រូវបានស្គាល់ច្បាស់ថាជាប្រភេទបាក់តេរីអេរ៉ូមូណាស ហៃត្រូហ្វីលឡា (*Aeromonas Hydrophila*)។ លទ្ធផលនេះបង្ហាញថា បាក់តេរីអេរ៉ូមូណាសគឺជាបាក់តេរីមួយប្រភេទដ៏សំខាន់នៅក្នុងចំណោមប្រភេទបាក់តេរីផ្សេងទៀត ដែលបានកំណត់ឃើញចេញពីការវិភាគនៅវិទ្យាស្ថានប៉ាស្ទ័រព្រោះបាក់តេរីប្រភេទនេះត្រូវបានស្គាល់ថា វាជាមូលហេតុធ្វើឱ្យសត្វផ្សោតចំនួន ៦ក្បាលស្លាប់ក្នុងអំឡុងពេល១៨ខែ កន្លងទៅនេះ។ បាក់តេរីនេះអាចបង្កឱ្យមានស្នាមជាំនៅត្រង់ករបស់សត្វផ្សោតព្រមទាំងបង្កឱ្យទៅជាដំបៅរលួយ។ ដូច្នេះការស្លាប់សត្វផ្សោតស្លាប់ទាំងអស់ដែលមានស្នាមជាំក្រមៅនៅត្រង់កអាចកំណត់ថាបណ្តាលមកពីប្រភេទបាក់តេរីអេរ៉ូមូណាស និងវាជាការកំរាមកំហែងដ៏ខ្លាំងចំពោះសត្វផ្សោតទន្លេមេគង្គ។ ថ្វីបើមានតែសំណាកសត្វផ្សោត ៦ ក្នុងចំណោមសំណាកសត្វផ្សោតសរុបចំនួន១១ក្បាលដែលបានវិភាគឃើញមានបាក់តេរីអេរ៉ូមូណាស ហៃត្រូហ្វីលឡា ចំណែកសំណាកសត្វផ្សោតចំនួន ៣ ផ្សេងនោះមិនឃើញមានវត្តមាន នេះដោយសារតែសាកសពសត្វផ្សោត ៣ ក្បាលនេះត្រូវបានបង្អកនៅសីតុណ្ហភាព ២០ អង្សាក្រោមសូន្យនៅមុនពេលធ្វើវះកាត់យកសំណាកសំរាប់វិភាគ។ បាក់តេរីប្រភេទនេះអាចធន់នឹងសីតុណ្ហភាពបានត្រឹមតែ ៤ អង្សា ក្រោមសូន្យប៉ុណ្ណោះ សីតុណ្ហភាពត្រជាក់ជាងនេះធ្វើឱ្យបាក់តេរីអេរ៉ូមូណាស ហៃត្រូហ្វីលឡាស្លាប់បាន ដូច្នេះការវិភាគរកសញ្ញាប្រភេទបាក់តេរីនេះគឺមិនអាចរកឃើញឡើយ។ សត្វផ្សោតទាំងបីក្បាលនេះមិនមានបាក់តេរីអេរ៉ូមូណាសឡើយ ព្រោះបាក់តេរីប្រភេទនេះត្រូវបានសំលាប់ក្នុងដំណាក់កាលនៃការបង្អកសាកសព។ ចំណែកសាកសពពីរផ្សេងទៀតនោះ គឺជាសាកសពរបស់សត្វផ្សោតវ័យជំទង់ដែលស្លាប់ដោយមានការចម្លងមេរោគចម្រុះ។ ចម្លើយនេះ ត្រូវគ្នាជាមួយលទ្ធផលដែលរកឃើញប្រភេទបាក់តេរីអេរ៉ូមូណាស ហៃត្រូហ្វីលឡា ដោយសារតែបាក់តេរីប្រភេទនេះជាប្រភេទបាក់តេរីឱកាសនិយមហើយជាទូទៅវាតែងបង្កជម្ងឺលើសត្វផ្សោតវ័យជំទង់ និងវ័យចំណាស់តែប៉ុណ្ណោះ។ ប្រសិនបើសត្វផ្សោតវ័យជំទង់អាចរស់បានដោយឆ្លងផុតពីការសំលាប់របស់បាក់តេរី អេរ៉ូមូណាស នៅក្នុងឆ្នាំទីមួយនោះប្រព័ន្ធការពារសិរាវរបស់ពួកវាអាចធន់បានទៅនឹងការលុកលុយរបស់បាក់តេរីប្រភេទនេះបាន។ ថ្វីបើ បាក់

តើអ្នកមូណាស មិនមែនជាឃាតករសំលាប់សត្វផ្សេងៗដទៃទាំងពីរនេះផ្ទាល់ក៏ដោយ តែអ្នកមូណាស បានធ្វើឱ្យប្រព័ន្ធការពារសរីរាង្គរបស់សត្វផ្សេងៗទាំងនេះចុះខ្សោយដែលបង្កឱ្យមេរោគ និងបាក់តេរីផ្សេងៗ ទៀតចូលមកសម្លាប់វាបាន។ លទ្ធផលវិភាគបញ្ជាក់ថាសត្វផ្សេងៗទាំង ២ ក្បាលនេះស្លាប់ដោយសារ ជម្ងឺក្នុងសួត (Pneumonia) និងបាក់តេរី ចម្រុះជាច្រើនទៀត។

ការរកឃើញបាក់តេរី អ្នកមូណាស គឺជាភ្នាក់ងារជម្ងឺមួយនោះ គឺមានការចាប់អារម្មណ៍យ៉ាងខ្លាំង ដោយសារវាបង្កឱ្យមានជម្ងឺដល់សត្វផ្សេងៗ តាមរយៈការធ្វើឱ្យប្រព័ន្ធការពារសរីរាង្គរបស់វាមិនមានដំណើរ ការប្រក្រតី។ ហេតុដូច្នេះហើយ វាមានកត្តាផ្សេងៗជាច្រើនដែលប៉ះពាល់ដល់ប្រព័ន្ធការពារសរីរាង្គរបស់ សត្វផ្សេងៗ និងនាំឱ្យសត្វផ្សេងៗទាំងនោះមានជម្ងឺឈានទៅរកក្តីស្លាប់។

ប្រព័ន្ធការពាររបស់សរីរាង្គ (ប្រព័ន្ធភាពស៊ាំ) គឺជាប្រព័ន្ធការពារដែលមានពីរធម្មជាតិរបស់ដង ខ្លួន ដើម្បីប្រឆាំងតទៅនឹងជម្ងឺដែលបង្កឡើងដោយភ្នាក់ងារចំលងផ្សេងៗ។ ប្រព័ន្ធការពារនេះមានតួនាទី បង្កើតកោសិកាគ្រាប់ឈាមសដែលមានលទ្ធភាពប្រឆាំងទៅនឹងជម្ងឺផ្សេងៗបង្កឡើងដោយភ្នាក់ងារចំលង។ សត្វផ្សេងៗដែលមានសុខភាពល្អ ប្រព័ន្ធការពារនេះមានកំលាំងគ្រប់គ្រាន់ ក្នុងការប្រឆាំងតទៅនឹងភ្នាក់ងារ ចំលងជម្ងឺឆ្លើរគ្រប់ប្រភេទហើយមិនបង្កឱ្យសត្វផ្សេងៗឈឺបានឡើយ។ យ៉ាងណាមិញចំពោះសត្វផ្សេងៗដែល ប្រព័ន្ធការពារសរីរាង្គត្រូវចុះខ្សោយអាស្រ័យដោយកត្តាផ្សេងៗដូចជា៖ ភាពតានតឹងក្នុងអារម្មណ៍ ជាតិពុល ផ្សេងៗ ការបំពុល ឬការរួមភេទក្នុងក្រុមតែមួយ ប្រព័ន្ធការពារសរីរាង្គរបស់សត្វផ្សេងៗទាំងនេះ មិនអាច ទប់ទល់នឹងភ្នាក់ងារចំលងជម្ងឺផ្សេងៗនោះបានឡើយ សត្វផ្សេងៗទាំងនោះត្រូវឈឺ ហើយអាចស្លាប់អាស្រ័យ ដោយជម្ងឺតែម្តង។

## **ការសិក្សាប្រភេទទី២**

ការវិភាគលើសំណាកសត្វផ្សេងៗទន្លេមេគង្គស្លាប់ចំនួន ២១ក្បាល បានរកឃើញកំរិតជាតិពុលគីមី សរីរាង្គ (Persistent Organochlorine Pesticides, POPs) ដូចជា DDT និង PCBs ព្រមទាំងកំរិត ជាតិហ្វីតដែលប៉ះពាល់ទៅនឹងប្រព័ន្ធការពារសរីរាង្គរបស់សត្វផ្សេងៗ។

ផ្អែកលើលទ្ធផលនៃការវិភាគបង្ហាញថា DDT នៅក្នុងស្រទាប់ខ្លាញ់ (មានចន្លោះពី ៤១០០ ទៅ ១២០០០ng/g) របស់សត្វផ្សេងៗក្បាលត្រឡោកទន្លេមេគង្គមានចំនួន១០ដង ខ្ពស់ជាង DDT មាននៅក្នុង ស្រទាប់ខ្លាញ់ (ចន្លោះពី ១១០០ ទៅ ៥០៥២ ng/g) របស់សត្វផ្សេងៗក្បាលត្រឡោកនៃទន្លេ ឈីលីកា (Chilika)។ កំរិត DDT នៅក្នុងឈាម (ចន្លោះ ពី ២២.១ ទៅ ២៤.៤ ng/g) បានកាត់បន្ថយការបង្កើត កោសិកាឈាមសរបស់ប្រព័ន្ធការពារសរីរាង្គនៃសត្វផ្សេងៗ។ បើទោះជាកំរិត DDT និង PCBs នៅក្នុង ចរន្តឈាមនៃសត្វផ្សេងៗក្បាលត្រឡោករបស់ប្រទេសកម្ពុជាមិនត្រូវបានវាស់ស្ទង់ក៏ដោយ ប៉ុន្តែជាការចាំ

បាច់ត្រូវយល់ឱ្យដឹងថាកំរិតជាតិគីមីពុលទាំងនេះ នឹងធ្វើឱ្យប្រព័ន្ធការពារសរីរាង្គដែលមានភាពស៊ាំមានការ ចុះខ្សោយ(វិភាគលើឈាមស្រស់របស់សត្វផ្សោតនៅក្នុងមន្ទីរពិសោធន៍)និងមានទំនាក់ទំនងជាមួយការ កើនឡើងនៃកំរិត DDT និង PCBs នៅក្នុងចរន្តឈាម ។ ជាលទ្ធផលបានបង្ហាញថា កំរិតជាតិគីមីពុល របស់សត្វផ្សោតក្បាលត្រឡោកនៃទន្លេ ឈីលីកា ទាបជាងឆ្ងាយ បើប្រៀបធៀបទៅនឹងកំរិតជាតិពុលដូច គ្នា (DDT និង PCBs)ដែលបានត្រូវពិនិត្យឃើញនៅក្នុងសំណាកស្រទាប់ខ្លាញ់របស់សត្វផ្សោតទន្លេនៅ ក្នុងប្រទេសកម្ពុជា ។

កំរិតជាតិបារីតនៅក្នុងសំណាកធ្វើមរបស់កូនផ្សោតចំនួន ៣ក្បាលក្នុងចំណោមកូនផ្សោតស្លាប់ចំនួន ៤ក្បាលនៃសត្វផ្សោតទន្លេមេគង្គនៃប្រទេសកម្ពុជា គឺស្ថិតនៅកំរិតខ្ពស់ជាង 1ppm(1μg/g)ដែលកំរិត 1ppm(1μg/g) នេះត្រូវបានចាត់ទុកថាជាកំរិតទាបបំផុត ដែលចាប់ផ្តើមនាំឱ្យប៉ះពាល់ដល់ភាពស៊ាំនៃ ប្រព័ន្ធសរីរាង្គ ។ ដូច្នេះ ជាតិបារីតពុលអាចចាត់ទុកថាមានលទ្ធភាពគំរាមកំហែងដល់កូនផ្សោតទន្លេមេគង្គ ទាំងឡាយផងដែរ ។ កំរិតជាតិបារីតទាំងនេះ បានប្រាប់ឱ្យដឹងថា សំណាកសត្វផ្សោតទាំង ៤ ដែលស្ថិតនៅ ក្នុងវ័យទើបនឹងកើតមានជាតិបារីតនៅក្នុងខ្លួនតាំងពីនៅក្នុងពោះមេរបស់ពួកវាម្ល៉េះ ដែលកំរិតជាតិបារីត នេះនឹងមានការកើនឡើងជាលំដាប់នៅក្នុងសរីរាង្គរបស់វា ។

លទ្ធផលការវិភាគពិនិត្យមើល DNA របស់សត្វផ្សោតចំនួន ៩ក្បាល ចេញពីក្រុមតែមួយបង្ហាញ ថាទំនាក់ទំនងហ្សេនរបស់ពួកវានៅក្បែរជិតគ្នា (Close genetic relationships) ក្នុងចំណោមសំណាក ទាំងអស់ដែលបានវិភាគ ។ នេះបញ្ជាក់ថា ហ្សេននេទិករបស់សត្វផ្សោតដែលចេញពីក្រុមតែមួយទាំងអស់ ស្ថិតនៅមានកំរិត (Limited gene pool) ដោយភាពខុសប្លែកគ្នានៃហ្សេនមានតិចតួចប៉ុណ្ណោះ ។ ការរួម ភេទរបស់សត្វផ្សោតក្នុងក្រុមតែមួយបែបនេះ អាចធ្វើឱ្យកំណត់មានការថយចុះ តាមរយៈការផលិតទឹក កាមមិនធម្មតានិងមិនមានគុណភាពព្រមទាំងកាត់បន្ថយចំនួនកូនផ្សោតទើបនឹងកើត និងមិនអាចរស់នៅ បានដល់ពេញវ័យ ។ ផលលំបាករយៈពេលវែងនៃការរួមភេទរបស់សត្វផ្សោតក្នុងក្រុមតែមួយ ជាពិសេស ចំពោះក្រុមសត្វផ្សោតដែលរស់នៅដាច់ដោយឡែកពីក្រុមដទៃទៀតក្នុងធម្មជាតិ និងការធ្លាក់ចុះចំនួនសរុប នៅក្នុងក្រុមរបស់ពួកវា អាចឈានទៅបាត់បង់ហ្សេននេទិកចម្រុះនៅក្នុងក្រុម ។ ការបាត់បង់ហ្សេននេទិក ចម្រុះអាចធ្វើឱ្យប្រព័ន្ធការពារសរីរាង្គសត្វផ្សោតនីមួយៗចុះខ្សោយ ដូច្នេះសត្វផ្សោតអាចងាយទទួលរងជំងឺ ឆ្លងផ្សេងៗដែលអាចឈានដល់ការវិនាសផុតពូជតែម្តង ។ ការរួមភេទរបស់សត្វផ្សោតនៅក្នុងក្រុមតែមួយក៏ អាចស្តែងចេញជាលទ្ធផលមួយដែលគេស្គាល់ថា " ការចុះអន់ថយផ្នែកកាយសម្បទារបស់កូន " (Inbreeding depression) ដែលធ្វើឱ្យប៉ះពាល់ និងមានគ្រោះថ្នាក់ដល់កូនផ្សោតទើបនឹងកើត ដោយ សារតែទំនាក់ទំនងផ្លូវភេទរវាងមេ និងបាចេញពីក្រុមតែមួយ ។ ប្រភេទសត្វខ្លះ អាចមិនមានផលប៉ះពាល់



ខ្លាំងដល់កូនដែលកើតចេញពីការការទំនាក់ទំនងរួមភេទក្នុងក្រុមតែមួយឡើយ ប៉ុន្តែទោះជាយ៉ាងណាការ រួមភេទក្នុងក្រុមតែមួយនាំទៅរកការចុះខ្សោយកម្លាំង សមត្ថភាព និងភាពឆ្លាតវៃ ហើយនាំឱ្យមាន ការផ្លាស់ប្តូរចរិតលក្ខណៈ លទ្ធភាពនៃការរស់នៅបានយូរ និងការបន្តពូជ ។

ការស្លាប់របស់សត្វផ្សោតក្បាលត្រឡោកវ័យចំណាស់នៃទន្លេមេគង្គ ត្រូវបានកត់ត្រាទុកជាឯក សារយ៉ាងល្អហើយមូលហេតុនៃការស្លាប់នេះគឺបណ្តាលមកពីការជាប់មងនេសាទ ។ ប៉ុន្តែក្នុងអំឡុងពេល ប៉ុន្មានឆ្នាំកន្លងមកនេះ ចំនួនកូនសត្វផ្សោតស្លាប់មានការកើនឡើងគួរឱ្យកត់សំគាល់ ហើយមូលហេតុនៃ ការស្លាប់គឺមិនទាន់ត្រូវបានបកស្រាវច្បាស់លាស់នៅឡើយ ។ ភស្តុតាងនាពេលថ្មីៗ ដែលបង្ហាញជូននៅក្នុង របាយការណ៍នេះ ប្រាប់ឱ្យដឹងថាអនាគតនៃការរស់រានមានជីវិតរបស់សត្វផ្សោតក្បាលត្រឡោកទន្លេមេគង្គ គឺកំពុងប្រឈមទៅនឹងគ្រោះថ្នាក់ អាស្រ័យដោយកត្តាជាច្រើនដែលគំរាមកំហែងយ៉ាងខ្លាំងក្លា ពិសេសទៅ លើកូនផ្សោត ។ កត្តាទាំងនេះ អាចសង្ខេបជូនដូចខាងក្រោម៖

**១) - ជម្ងឺ**

- ក) - អេរ៉ូមូណាស ហៃត្រូហ្វីលឡា (*Aeromonas Hydrophila*)
- ខ) - ជម្ងឺផ្សេងៗដែលបង្កឡើងដោយប្រភេទបាក់តេរីឱកាសនិយម

**២) - ជាតិពុលដែលមាននៅក្នុងបរិស្ថាន**

- ក) - ជាតិពុលគីមីសរីរាង្គ (DDT & PCBs)
- ខ) - ជាតិបារីត

**៣) - ការចុះអន់ថយផ្នែកកាយសម្បទារបស់កូន (Inbreeding depression)**

- ក) - ហ្សែននេទិកចម្រុះមានកំរិតទាប

កំរិតជាតិពុលគីមីសរីរាង្គ (DDT) និងជាតិបារីតដែលបានរកឃើញនៅក្នុងសំណាកសត្វផ្សោតទន្លេ មេគង្គគឺបង្កឱ្យមានផលប៉ះពាល់ដល់ភាពបន្ស៊ាំនៃសរីរាង្គរបស់សត្វផ្សោត ។ គេជឿថាកំរិតជាតិគីមីពុលនៅ ក្នុងបរិស្ថានមានការផលប៉ះពាល់ទៅលើប្រព័ន្ធការពារសរីរាង្គរបស់សត្វផ្សោត ដែលនាំឱ្យប្រព័ន្ធការពារនេះ គ្មានលទ្ធភាពការពារប្រឆាំងទៅនឹងប្រភេទបាក់តេរីដែលជាភ្នាក់ងារចំលងជម្ងឺផ្សេងៗ ដូចជា អេរ៉ូមូណាស ហៃត្រូហ្វីលឡា ។ ជាងនេះទៅទៀតនោះ ហ្សែនដែលមានលក្ខណៈកំរិតនៅក្នុងសត្វផ្សោតនីមួយៗរបស់ ក្រុមមួយ អាចធ្វើឱ្យមានការចុះអន់ថយផ្នែកកាយសម្បទារបស់កូន ប្រព័ន្ធការពារសរីរាង្គខ្សោយចុះ និង ការកើនឡើងចំនួនស្លាប់របស់កូនផ្សោត ។ គំនូសតាងខាងក្រោមសង្ខេបពីអត្ថន័យរួម៖

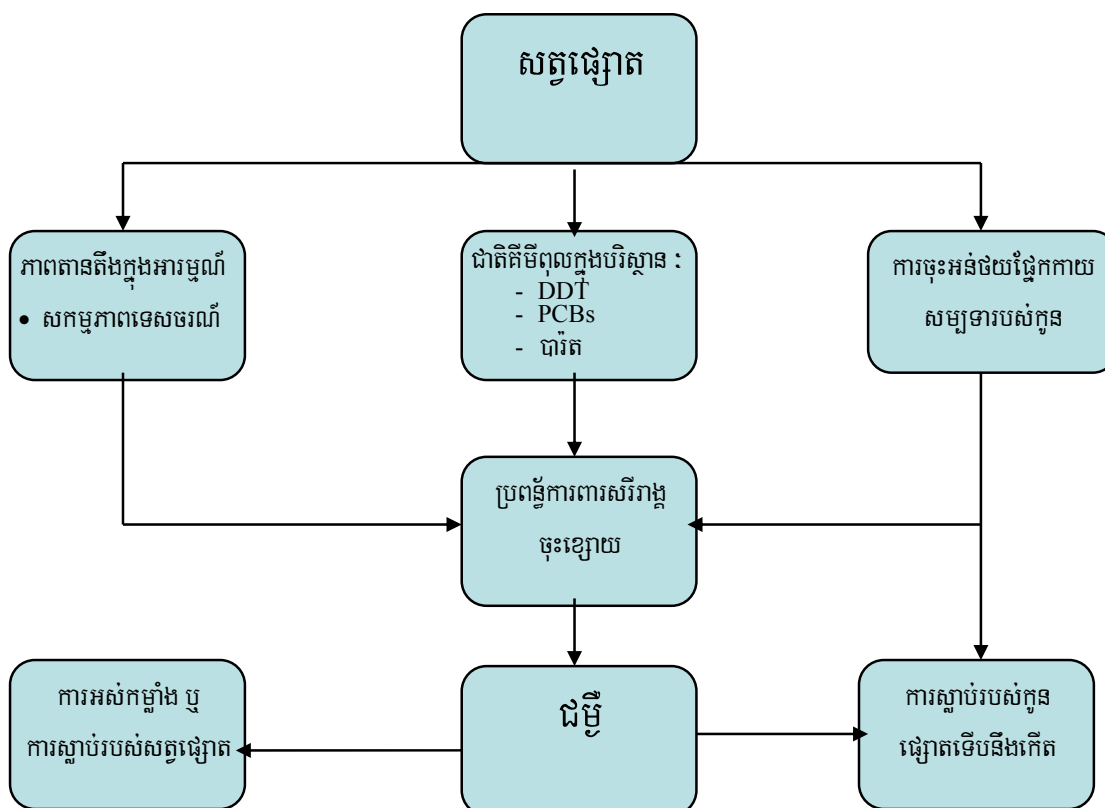
### ផលប៉ះពាល់ចេញពីបញ្ហាប្រឈមនីមួយៗចំពោះសត្វផ្សោត

សត្វផ្សោត + DDT > 22.1 ng.g => ប្រពន្ធការពារសិរីរាង្គចុះខ្សោយ  
 សត្វផ្សោត + PCBs => ប្រពន្ធការពារសិរីរាង្គចុះខ្សោយ  
 សត្វផ្សោត + ជាតិបារីត (Hg) > 1ppm => ប្រពន្ធការពារសិរីរាង្គចុះខ្សោយ  
 សត្វផ្សោត + Inbreeding Depression (ID) => ប្រពន្ធការពារសិរីរាង្គចុះខ្សោយ + អាត្រាស្លាប់នៃកូន  
 ផ្សោតទើបនឹងកើតកើនឡើង ។

### ផលប៉ះពាល់ចេញពីបញ្ហាប្រឈមរួមបញ្ចូលគ្នាចំពោះសត្វផ្សោត

សត្វផ្សោត + DDT + PCBs + Hg + ID => ប្រពន្ធការពារសិរីរាង្គចុះខ្សោយខ្លាំង + អាត្រាស្លាប់នៃកូន  
 ផ្សោតទើបនឹងកើត កើនឡើងខ្លាំង ។  
 ប្រពន្ធការពារសិរីរាង្គរបស់ផ្សោតចុះខ្សោយកាន់តែខ្លាំង => ជម្ងឺ និងអាចស្លាប់ ។

បញ្ហាប្រឈមទាំងនេះត្រូវបានពិពណ៌នាតាមរយៈក្រាហ្វិកដូចខាងក្រោម៖



## **សន្និដ្ឋាន**

ផ្អែកលើលទ្ធផលនៃការវិភាគនេះ ជាតិពុលគីមីសរីរាង្គ (ពិសេស DDT និង PCBs) ជាតិប៉ារ៉ា និង កំរិតហ្សេនេទិកចម្រុះ ត្រូវបានរកឃើញថាជាមូលហេតុចម្បងនៃការគំរាមកំហែងចំពោះសត្វផ្សោតទន្លេមេគង្គ។ លើសពីនេះទៅទៀត ការវិភាគនៅវិទ្យាស្ថានប៉ាស្ទ័រក្នុងប្រទេសកម្ពុជា បានរកឃើញប្រភេទជម្ងឺដែលបង្កឡើងដោយពពួកបាក់តេរី ពិសេសបាក់តេរី អេរីយូណាស ហៃត្រូហ្វីលឡាដែលជាការគំរាមកំហែងបន្ថែមមួយទៀតលើសត្វផ្សោតក្បាលត្រឡោកទន្លេមេគង្គ។

## 1. EXECUTIVE SUMMARY

The Mekong River Irrawaddy dolphins (*Orcaella brevirostris*) are one of three Riverine Irrawaddy dolphin populations, all classed as Critically Endangered. The current population abundance estimate of the Mekong population is 66-86 individuals. Since 2003 there have been 88 dolphin deaths recorded, making this population of freshwater Irrawaddy dolphins the likely the most threatened of all the populations, and on the verge of extinction.

This report documents the results obtained from dead dolphin samples from two different studies.

**Study 1:** Microbiological testing has been carried out on samples collected from 11 dead dolphins since 2007. This testing was done in Cambodia at a French medical laboratory, the Institute Du Pasteur, in Phnom Penh.

**Study 2:** Twenty-one samples collected from necropsies conducted between 2004 and 2006 were selected and shipped overseas to various diagnostic laboratories in the USA and Canada. Samples were submitted for various tests including

- PCR Pathogen (disease) screening
- Histopathology (microscopy)
- Toxicology (environmental contaminants)
- Heavy Metal (Mercury and Selenium)
- Dioxin/Furan analysis
- Genetic analysis

**Study 1:** The results of the microbiological testing carried out at Pasteur Institute in Phnom Penh has identified **disease** as a cause of death in 10 out of the 11 animals submitted for testing.

A significant proportion of dead dolphins have very obvious neck-lesions that look like blue / black bruising when the dolphins are first collected. During the necropsies, these neck lesions look very similar to gangrene. One of the bacterial agents cultured from biopsy of the neck lesion is known as *Aeromonas hydrophila*. This appears to be the most significant of all the disease agents identified from the testing at Pasteur Institute as it has been identified as the cause of death in six animals over the past 18 months. This bacterium can cause the sort of neck-lesions we are seeing in the dolphins, as well as result in gangrene lesions. Therefore, from the number of dead dolphins with neck lesions present this disease agent is now considered the biggest threat to the Mekong dolphin population. Although only six of the eleven dolphins tested had the *Aeromonas* bacteria present, there were 3 carcasses that were stored frozen at -20°C prior to testing that did not have *Aeromonas*. The freezing process kills the *Aeromonas* bacterium, which can only survive cold temperatures down to -4°C, so it would not be present when they culture the tissue samples from the frozen carcasses. It is therefore a possibility that these three dolphins did have *Aeromonas*, but the bacteria were killed in the freezing process. The other two remaining carcasses were both juveniles, with mixed infections. This fits with the findings of *Aeromonas*, as it is an opportunistic bacteria that usually only causes disease in the young and the old. If juveniles survive their first year without dying from *Aeromonas* it is likely that their immune system was strong enough to fight off this bacteria. However although *Aeromonas* did not kill these two juveniles, they both had pneumonia (lung infections) and had mixed infections with many bacteria, indicating that their immune system had become weak.

The finding of *Aeromonas* as a disease agent is also very interesting as it only causes disease in the host (in this case it's the dolphins), when the immune system is not working correctly. Therefore there must be factors affecting the dolphin's immune system for this disease to result in death.

An immune system is the body's natural defence against disease causing agents. The immune system produces white blood cells that are capable of fighting disease causing agents (pathogens). In a healthy dolphin the immune system is strong enough to fight off most pathogens, and not allow the dolphin to become sick or debilitated from disease. In a dolphin in which the immune system is weakened by stress, immuno-toxic contaminants, pollution, or genetic inbreeding, the dolphins' immune system cannot fight the pathogens and so the dolphin gets sick and debilitated, and may even die from these diseases.

**Study 2:** Analysis on samples from 21 dead dolphins have found levels of Persistent Organochlorine Pesticides (POPs) such as DDT and PCBs as well as Mercury levels that are immuno-toxic (toxic to the dolphins' immune system).

From the analysis we have found that the blubber levels of DDT were ten times higher in the Cambodian Irrawaddy dolphins (with a range of 4100-12000 ng/g) when compared to that found in Irrawaddy dolphins in Chilka Lake (with a range of 1100-5052ng/g). DDT blood levels of 22.1-24.4 ng/g have been found to reduce the white cell production of dolphins' immune system. Although circulating (blood) levels of DDT and PCBs were not measured in the Cambodian Irrawaddy dolphins, it is important to acknowledge that these contaminants have shown a reduced *in vitro* immune response (using fresh dolphin blood in the laboratory) associated with increasing levels of DDT and PCBs in circulating blood, at levels far lower than those reported in the blubber samples of the Cambodian dolphins.

The liver mercury concentrations from three out of the four dolphin calves sampled in Cambodian Irrawaddy dolphins were above 1ppm (1µg/g), which is considered the lowest threshold for being immuno-toxic. Thus mercury contamination should be considered a potential threat to the Mekong River Irrawaddy Dolphin calves. These levels of Mercury contamination are even more significant given that all four dolphins were neonatal calves, and that mercury accumulation is age-dependant, with higher levels accumulating over time due to bioaccumulation.

The results of the DNA testing of 9 individuals from the population show close genetic relationships between all the animals tested, suggesting a limited gene pool in

this population with little genetic variation. Close inbreeding can cause a significant reduction in reproduction success with abnormal sperm production and increased infertility, as well as a reduction in the number of calves born and reduced calf survival. The long-term consequence of inbreeding, particularly with a naturally isolated, declining wild population may result in the loss of genetic diversity. This loss in genetic diversity could result in each animal's immune system becoming weakened, thus rendering the population susceptible to a potential epidemic that could be fatal to the entire population. Inbreeding can also result in what is known as "inbreeding depression", which is the production of inherited deleterious traits in calves as a consequence of the close relationship between their parents. Some species cope well with inbreeding without the deleterious effects; however inbreeding depression preferentially reduces the fitness of a population, by altering characteristics associated with an individual's probability of survival, and reproduction.

It has been well documented that adult Mekong Irrawaddy dolphins have died as a result of accidental entanglement in gill nets. However, in recent years there has been an increasing number of deaths of juvenile dolphins, the cause of which had been a mystery up until now. Emerging evidence described in this report suggests that the future survival of the Mekong Irrawaddy dolphin population is at risk, due to a combination of factors that particularly threaten the young dolphins. These factors can be summarized as:

**1. Disease**

- a. *Aeromonas hydrophila*
- b. Other opportunistic bacterial diseases

**2. Environmental contaminants**

- a. POPs (DDT & PCBs)
- b. Mercury

**3. Inbreeding depression**

- a. Low genetic diversity

As the levels of DDT and Mercury found in the Mekong dolphins are immuno-toxic in their own right, it is believed that the immuno-toxic levels of environmental contaminants are having a synergistic or additive effect on the dolphins' immune system, rendering the immune system defenceless against pathogenic (disease

causing) bacteria such as *Aeromonas hydrophila*. In addition the limited genetics amongst individual dolphins in this population may be resulting in inbreeding depression, further weakening the dolphins' immune systems, and adding to the increased number of dolphin calf deaths. See diagrams below that summarises this concept:

The effect of each individual threat on the dolphin:

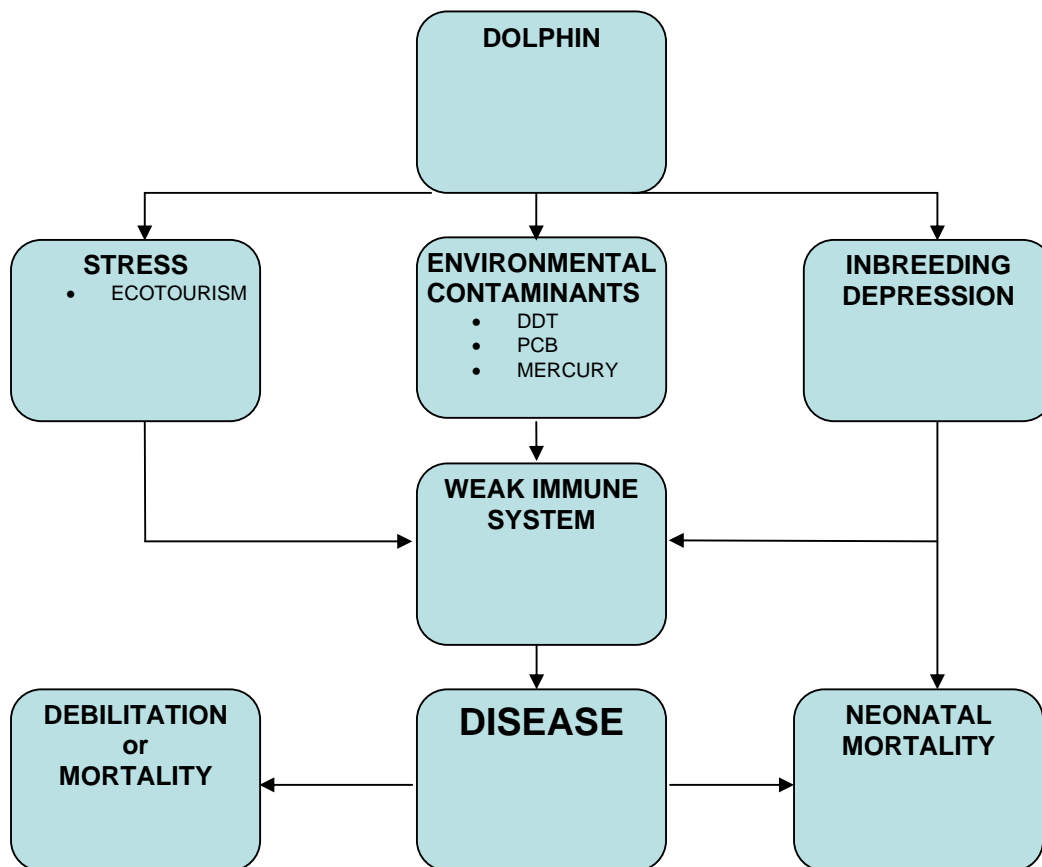
Dolphin + DDT >22.1 ng/g	⇒ ↓ Immune function
Dolphin + PCBs	⇒ ↓ Immune function
Dolphin + Mercury (Hg) >1ppm	⇒ ↓ Immune function
Dolphin + Inbreeding Depression (ID)	⇒ ↓ Immune function + ↑ Neonatal mortality

The effect of synergy of all the threats on the dolphin:

Dolphin + DDT + PCBs + Hg + ID	⇒ ↓↓↓↓ Immune function + ↑ Neonatal mortality
↓↓↓↓ Dolphins' Immune function	⇒ Disease and likely death



These threats are depicted in the following flow chart:



### **Conclusion**

From the results from these analysis, contamination with POPs particularly DDT and PCBs, as well as mercury, together with limited genetic diversity have all been identified as viable threats to the Mekong River dolphin population. In addition further analysis carried out within Cambodia at Pasteur institute has identified bacterial diseases particularly *Aeromonas hydrophila* as an additional threat to this population.

Please refer to Recommendations on page 46.

## 2. INTRODUCTION

In the last 50 years of the previous century it became clear that human pressures on the environment presented a serious threat to the viability of many species. The current rate of loss of species is thought to be unprecedented and the Endangered Species Act mandates that in addition to stopping most activities causing the decline of an endangered species, a management plan must be produced that will rehabilitate the species by bringing its population size above the threshold of endangerment. River dolphins are amongst the world's most seriously endangered species. Irrawaddy dolphins (*Orcaella brevirostris*) inhabiting the Mekong River in Cambodia and Southern Laos were red Listed as "Critically Endangered by the World Conservation Union (IUCN) in 2004. The conservation of these dolphins requires an understanding of biogeographical patterns, community structure, population dynamics and individual behaviour, and a sound understanding of health issues that affects this population.

Preliminary mark and recapture estimates using photo-identification analysis, carried out in 2007, estimates the population abundance at 71 individuals (95%CI 66-86) (Dove et al., 2008). Since 2003 there have been 88 confirmed dolphin deaths, of which 56 (68%) have been neonatal calves. In 2006 there were 19 confirmed mortalities of which 16 were neonatal calves, and 14 mortalities in 2007 of which 12 were neonatal calves. With such a high and unsustainable mortality rate, and marginal recruitment due to the large proportion of calves dying, the Mekong Population is likely to be the most critically threatened population of freshwater Irrawaddy dolphins. With this population in serious decline, they face extinction in the near future, if immediate conservation action is not taken.

The first step in the conservation of this species is to diagnose the cause of mortality in the dolphins. Once the cause has been established we can start working on conservation strategies to recover this population, and save them from extinction. It is important to realize the role the infectious diseases, particularly Emerging Infectious Diseases (EIDs) can play with regard to population dynamics as well as population declines and in some cases even result in population extinctions. Even more importantly is acknowledging the links associated with anthropogenic environmental changes, and the emergence of these EIDs in humans and wildlife populations. In

long-lived species like Irrawaddy dolphins, infectious diseases that have fatal outcomes, affect fecundity, or reproductive success, could significantly impact the population size and viability. Similarly, environmental contaminants or genetic inbreeding that are capable of reducing the dolphins' immune capacity, rendering them susceptible to infectious disease, could also significantly affect the long-term survival of a critically endangered population.

### **3. METHODOLOGY**

#### **3.1. Necropsies**

Complete and standardized post-mortem (necropsy) examination of all dead dolphins from this critically endangered population serves as an excellent first step in gathering information about infectious diseases in the population. Post mortems also allow us to assess environmental contaminant levels that may pose a threat to the health status of this population. Using sentinel animals such as the Mekong River Irrawaddy dolphins to collect base-line disease surveillance information, will allow the detection of potentially hazardous environmental conditions to be identified.

Baseline data can be used in comparisons with the same population at a future date to determine the effects of various disturbances such as eco-tourism, dam development, and habitat loss, as well as for comparisons between different populations. This will be valuable in determining appropriate conservation management techniques for individual populations.

#### **3.2. Epidemiology in disease surveillance**

The inter-relationships that underlie the emergence of infectious diseases (EIDs) are complex and often multi-factorial. For example human encroachment into the dolphins' environment brings the dolphins into contact with humans and domestic animals, which may facilitate disease "spill over". In addition, contaminants from anthropogenic (human) activities may result in toxicosis (eg. heavy metals), or immune suppression (eg. Persistent Organochlorine Pesticides and Mercury), which can alter the dolphins' normal defence systems to ward off disease. Other environmental changes such as climate change may facilitate pathogen (disease agents) emergence, or transportation of pathogens or their vectors to a naïve environment. Similarly, global travel of humans may facilitate introduction of novel pathogens to a new environment. Thus the study of EIDs in wild dolphin populations are difficult as many of these factors may act synergistically, and so the appropriate use of epidemiological principles needs to be applied when investigating diseases in wildlife populations.

### **3.3. Sample analysis**

This study will report on sample analysis from eleven dead dolphins collected between 2007 and 2009 and sent to Pasteur Institute in Phnom Penh for microbiological analysis. In addition samples from twenty-one dead dolphins collected from necropsies (see Gilbert and Beasley 2005) conducted between 2004 and 2006, were selected and shipped overseas to various diagnostic laboratories in the USA and Canada in 2006. These samples were submitted for PCR pathogen screen, histopathology, toxicology, heavy metal, dioxin/furan and genetic analysis. Due to permit problems with overseas shipments from Canada to the USA, and a backlog of samples due to hurricane Katrina at one of the laboratories, the results from these analyses were only received at the end of March 2008.

See Table 1 below for a full list of samples shipped in 2006, and Table 2 for the various diagnostic analyses carried out on each sample.

**Table 1: Details of the tissues exported in 2006**

<b>Specimen Code</b>	<b>Species</b>	<b>Common name</b>	<b>Age</b>	<b>Blubber<sup>†</sup></b>	<b>Lung<sup>†</sup></b>	<b>Liver<sup>†</sup></b>	<b>Kidney<sup>†</sup></b>	<b>Stomach<sup>†</sup></b>	<b>Brain<sup>†</sup></b>	<b>Formalin*</b>
OBRE03-11/06	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Adult	1						
OBRE03-02/08	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Adult	1						
OBRE04-06/06	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Adult	1						
OBRE04-08//02	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Adult	1			2		1	
OBRE04-24/02	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1						
OBRE04-20/03	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1	1	1	1	1		
OBRE04-28/09	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1	1	1	1			1
OBRE04-09/11	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1			2
OBRE04-22/11	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf							1
OBRE05-20/01	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf							1
OBRE05-09/03	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1		1	
OBRE05-19/03	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf							2
OBRE05-24/05	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf							1
OBRE05-10/12	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf							1
OBRE06-08/01	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Juvenile	1	1	1	1			
OBRE06-13/01	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1			3
OBRE06-14/01A	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1			
OBRE06-14/01B	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1			1
OBRE06-01/02	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1	1	1	1	1		
OBRE06-13/02B	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1		1	1			2
OBRE06-15/02A	<i>Orcaella brevirostris</i>	Irrawaddy dolphin	Calf	1	1	1	1	1		

Numbers, refer to number of individual tissues (†) or bottles (\*) within shipment

**Table 2: Tests carried out on each sample**

Specimen Code	Age	Blubber	Lung	Liver	Kidney	Stomach	Brain	Formalin	Diagnostic Analysis
OBRE03-11/06	Adult	1							
OBRE03-02/08	Adult	1							
OBRE04-06/06	Adult	1							Dioxins
OBRE04-08/02	Adult	1			2		1		Dioxin, PCR
OBRE04-24/02	Calf	1							Dioxin
OBRE04-20/03	Calf	1	1	1	1	1			PCR
OBRE04-28/09	Calf	1	1	1	1			1	Dioxin, PCR, Histopathology
OBRE04-09/11	Calf	1		1	1			2	Histopathology, Trace minerals, POPs
OBRE04-22/11	Calf							1	Histopathology
OBRE05-20/01	Calf							1	Histopathology
OBRE05-09/03	Calf	1		1	1		1		Trace Minerals, POPs
OBRE05-19/03	Calf							2	Histopathology
OBRE05-24/05	Calf							1	Histopathology
OBRE05-10/12	Calf							1	
OBRE06-08/01	Juvenile	1	1	1	1				PCR, POPs
OBRE06-13/01	Calf	1		1	1			3	Histopathology, Trace minerals, POPs
OBRE06-14/01A	Calf	1		1	1				Dioxin, PCR
OBRE06-14/01B	Calf	1		1	1			1	Histopathology
OBRE06-01/02	Calf	1	1	1	1	1			PCR
OBRE06-13/02B	Calf	1		1	1			2	Histopathology, Trace minerals, POPs
OBRE06-15/02A	Calf	1	1	1	1	1			

## 4. RESULTS

### 4.1. Microbiological Testing at Pasteur Institute

In 2007, the first six dead dolphins in 2007 (CID 07 001- CID 07 006) were all buried by the river-guards, and most were eventually recovered for examination. However these carcasses were badly decomposed and so microbiological testing only commenced on the 7<sup>th</sup> dead dolphin in 2007. Since this time eleven dead dolphins have had samples submitted to Pasteur Institute for culturing bacteria. The following Table displays the results from Pasteur Institute.

**Table 3: Bacterial Culture Results from Pasteur Institute Phnom Penh.**

Specimen number	Age	Neck Lesion	CS	Date Reported	Pasteur (Microbiology) Results
CID 07 007	Calf	yes	3	15-03-07	<i>Aeromonas hydrophila</i>
CID 07 009	Calf	yes	2	9-04-07	<i>Aeromonas hydrophila</i> <i>Morganella morganii</i>
CID 07 011	Adult	Yes	3	14-06-07	<i>Klebsiella Pneumonia</i> <i>Streptococcus B haemolytica</i> infection,
CID 07 012	Calf	Yes	2	13-07-07	<i>Aeromonas hydrophila</i> <i>Plesiomonas shigelloides</i>
CID 07 014*	Calf	Yes	2	2-01-08	<i>Plesiomonas shigelloides</i>
CID 08 001*	Calf	Yes	2	1-01-08	<i>Plesiomonas shigelloides</i>
CID 08 002	Calf	Yes	3	4-03-08	<i>Aeromonas hydrophila</i> <i>Plesiomonas shigelloides</i>
CID 08 003*	Juvenile	Yes	3	26-05-08	No bacteria were cultured
CID 08 005	Adult	Yes	2	20-07-08	<i>Aeromonas hydrophila</i> <i>E coli</i> <i>Streptococcus B haemolytica</i> group C infection
CID 09 001	Juvenile	Yes	3	5-01-09	<i>Edwardsiella tarda</i> <i>Streptococcus B haemolytica D</i> <i>Proteus mirabilis</i> & <i>P. vulgaris</i>
CID 09 002	Adult	No	2	8-01-09	<i>Aeromonas hydrophila</i> <i>E.coli</i>

CS= Condition Score

\*Carcasses were frozen at -20degrees Celsius for up to a month until permission was given to perform necropsy. Freezing kills most bacteria, particularly *Aeromonas hydrophila*, that can only survive up to -4 degrees Celsius.



#### 4.2. Polymerase Chain Reaction (PCR) Pathogen Screen

Introduction of a highly virulent transmissible pathogen in such a small population of Irrawaddy river dolphins has the potential to catastrophically affect the long-term viability of the population. Six specimens were submitted to the Animal Health Centre, in Canada on the 25<sup>th</sup> of September 2006 for PCR pathogen screening for the following 5 pathogens; Mollicutes (M), Herpes virus (H), Erysipelothrix (Er), Morbillivirus (Mb) and Toxoplasma (Tg), See Table 12 below.

**Table 4: Irrawaddy Dolphin Sample Pathogen screen**

Dolphin Sample	Age	Pathogen Screen	Result
OBRE04-08/02	Adult	M, H, Er, Mb, Tg	Negative for all pathogens screened
OBRE04-20/03	Calf	M, H, Er, Mb, Tg	Negative for all pathogens screened
OBRE04-28/09	Calf	M, H, Er, Mb, Tg	Negative for all pathogens screened
OBRE06-01/02	Calf	M, H, Er, Mb, Tg	Negative for all pathogens screened
OBRE06-08/01	Calf	M, H, Er, Mb, Tg	Negative for all pathogens screened
OBRE06-14/01A	Calf	M, H, Er, Mb, Tg	Negative for all pathogens screened

The results from the Animal Health Centre for molecular diagnostic analysis using PCR found all 6 samples to be negative for the following disease agents:

- Mollicutes (M)
- Herpesvirus (H)
- *Erysipelothrix rhusiopathiae* (Er)
- Morbillivirus (Mb)
- *Toxoplasma gondii* (Tg)

#### 4.3. Histopathology

There was nothing definitive to report histologically as all tissue samples were moderately to markedly autolyzed (tissue breakdown). Many tissues had intravascular bacteria (bacteria within blood vessels), but this could also be due to post-mortem overgrowth, particularly when they are present without inflammatory changes or

vasculitis (inflammation of the blood vessels) as may have been the case in these samples. The degree of post-mortem autolysis hindered thorough histologic examination of many tissues, however there was no inflammatory infiltrate seen anywhere. Many tissues also had large gas bubbles present, consistent with overgrowth of clostridial organisms, again most likely post-mortem change. When sections of the neck lesions were examined histologically there was no evidence of haemorrhage or other changes in the skin, even in areas where the pathologist cut in to include grossly normal and abnormal regions. These tissues simply appeared severely autolyzed.

#### 4.4. Heavy Metal Analysis

Table 5 and 6 show the mercury and selenium results from the Irrawaddy dolphin samples sent to North Dakota Laboratory. The elemental concentrations in these tissues were reported on a wet-weight micro molar ( $\mu\text{mol}$ ) basis to enable comparison. Table 6 shows these results converted to  $\mu\text{g/g}$  for comparison with studies in the literature. All samples that were read in duplicate were averaged; with the average value reported in Table 5.

**Table 5: Mercury and Selenium results from the Irrawaddy dolphin in  $\mu\text{mol/g}$ -wet weight**

<b>Irrawaddy Dolphin</b>	<b>Age</b>	<b>Tissue</b>	<b><math>\mu\text{mol Hg/g}</math></b>	<b><math>\mu\text{mol Se/g}</math></b>	<b>Hg:Se</b>	<b>Se:Hg</b>
OBRE 04-09/11	Calf	Liver	4.27	12.36	0.35	2.9
OBRE 05-09/03	Calf	Liver	6.43	16.16	0.40	2.51
OBRE 06-13/01	Calf	Liver	7.7	44.61	0.17	5.79
OBRE 06-13/02B	Calf	Liver	6.51	16.72	0.39	2.57
<b>Mean</b>			<b>6.23</b>	<b>22.46</b>	<b>0.33</b>	<b>3.44</b>
<b>SD</b>			<b>1.43</b>	<b>14.89</b>	<b>0.11</b>	<b>1.58</b>
OBRE 04-09/11	Calf	Kidney	1.41	11.82	0.12	8.39
OBRE 05-09/03	Calf	Kidney	1.54	11.46	0.13	7.46
OBRE 06-13/01	Calf	Kidney	1.52	8.5	0.18	5.58
OBRE 06-13/02B	Calf	Kidney	1.56	8.92	0.17	5.73
<b>Mean</b>			<b>1.51</b>	<b>10.17</b>	<b>0.15</b>	<b>6.79</b>
<b>SD</b>			<b>0.07</b>	<b>1.71</b>	<b>0.03</b>	<b>1.37</b>
OBRE 04-09/11	Calf	Blubber	0.33	3.63	0.09	11.04
OBRE 05-09/03	Calf	Blubber	0.14	3.34	0.04	24.51
OBRE 06-13/01	Calf	Blubber	0.31	3.02	0.10	9.6
OBRE 06-13/02B	Calf	Blubber	0.24	2.66	0.09	11.11

<b>Mean</b>			<b>0.25</b>	<b>3.16</b>	<b>0.08</b>	<b>14.06</b>
<b>SD</b>			<b>0.09</b>	<b>0.42</b>	<b>0.03</b>	<b>7.00</b>
OBRE 05-09/03	Calf	Brain	1.02	4.26	0.61	1.64

**Table 6: Mercury and Selenium results from the Irrawaddy dolphin in µg/g-wet weight**

<b>Irrawaddy Dolphin</b>	<b>Age</b>	<b>Tissue</b>	<b>µg Hg/g</b>	<b>µg Se/g</b>	<b>Hg:Se</b>	<b>Se:Hg</b>
OBRE 04-09/11	Calf	Liver	0.86	0.98	0.88	1.14
OBRE 05-09/03	Calf	Liver	1.29	1.28	1.01	0.99
OBRE 06-13/01	Calf	Liver	1.54	3.52	0.44	2.28
OBRE 06-13/02B	Calf	Liver	1.31	1.32	0.99	1.01
OBRE 04-09/11	Calf	Kidney	0.28	0.93	0.30	3.30
OBRE 05-09/03	Calf	Kidney	0.31	0.90	0.34	2.93
OBRE 06-13/01	Calf	Kidney	0.30	0.67	0.45	2.20
OBRE 06-13/02B	Calf	Kidney	0.31	0.70	0.44	2.25
OBRE 04-09/11	Calf	Blubber	0.07	0.29	0.23	4.33
OBRE 05-09/03	Calf	Blubber	0.03	0.26	0.11	9.39
OBRE 06-13/01	Calf	Blubber	0.06	0.24	0.26	3.83
OBRE 06-13/02B	Calf	Blubber	0.05	0.21	0.23	4.36
OBRE 05-09/03	Calf	Brain	0.20	0.34	0.61	1.64

The selenium concentration in one liver sample from OBRE 06-13/01 was approximately 3-fold higher than in the other samples. This sample was double-checked by the laboratory and the high value reproduced thus verifying the authenticity of this elevated value. Mercury and selenium tissue concentrations were otherwise observed to be quite consistent between samples. Blanks, controls, and repeat readings were all consistent and in the normal range.

#### **4.5. Dioxin Analysis**

To assess the level of toxicity of dioxins in stranded Mekong Irrawaddy dolphins (*Orcaella brevirostris*), seven Polychlorinated-dibenzo-p-dioxin congeners (PCDDs) and ten polychlorinated dibenzofuran congeners (PCDFs) were analysed in blubber obtained

from five individuals. Samples were collected from two adult and three neonatal calf specimens. Among the PCDDs and PCDFs, the majority of the chlorine-substituted congeners were measured below the detection limit of 0.245 pg/g-wet wt, including the most toxic congeners 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD. Some other congeners did not meet the method criteria (EPA Method 1613) by either deviation in retention time, ion abundance ratio or peak shape.

**Table 7: Average results for 5 dolphins sampled for Dioxins and Furans**

Test	Toxic Equivalents	Units
Dioxins	4.11E-4 - 0.424	pgTEQ/g wet wt.
Furans	0.356 – 0.424	pgTEQ/g wet wt.

From Table 7, the 2,3,7,8-TCDD toxic equivalents (TEQs) of Dioxins: PCDDs/DFs that were able to be analysed, were found to range between 4.11E-4 and 0.424 pgTEQ/g wet wt. The total furan analysis had a TEQ-level had a range of 0.356 to 0.424 pgTEQ/g wet wt. Higher levels of hepta- and octa-CDDs, and TCDF were measured in the blubber samples, than any other congeners. No differences in the presence of dioxin congeners were observed between adult and calf specimens with the exception of two calves in which low TEQ levels of 1,2,3,4,7,8,9-HpCDF (0.00265 pgTEQ/g wet wt) and 1,2,3,4,7,8-HxCDD (0.0287 pgTEQ/g wet wt) that were measured in the blubber samples.

#### **4.6. Persistent Organochlorine Pesticides (POPs)**

Total lipids and lipid classes in the blubber of the five Irrawaddy dolphins are shown in Table 8. The results of the lipid class analysis showed that the lipids in three of the

blubber samples were composed mostly or completely of triglycerides, indicating that the samples were reasonably fresh, and that little or no sample degradation had occurred prior to analysis. Two blubber samples contained higher proportions (17.1-23.8%) of free fatty acids, indicative of some sample degradation. The total lipid content in the blubber samples ranged from 7.4 to 28.2 percent.

**Table 8: Total lipid and lipid classes<sup>a</sup> in blubber of Irrawaddy dolphins collected in Cambodia 2004-2006**

Animal	Age	Lipid %	SALE (% of Total)	TG (% of Total)	FFA (% of Total)	CHOL (% of Total)	PL (% of Total)
04-09/11	Calf	7.7	1.8	86.2	1.2	0.0	0.0
05-09/03	Calf	28.2	3.7	71.2	23.8	0.0	0.0
06-08/01	Juvenile	18.8	0.0	77	17.1	0.0	0.0
06-13/01	Calf	12.3	0.0	100.0	0.0	0.0	0.0
06-13/02B	Calf	13.8	0.0	100.0	0.0	0.0	0.0

<sup>a</sup> Lipid % and lipid classes are measured by TLC-FID to precision of 0.1%

SALE = steric acid laurel (wax) esters; TG = triglycerides; FFA = free fatty acids; CHOL = cholesterol; PL = phospholipids

Concentrations of POPs in blubber samples from Irrawaddy dolphins in Cambodia are shown in Table 9.

**Table 9: Concentrations (ng/g, Lipid) of POPs measured in blubber of Irrawaddy dolphins collected in Cambodia 2004-2006**

Animal	Class	HCB	ΣHCHs	ΣCHLDs	ΣDDTs	ΣPCBs	ΣPBDEs
04-09/11	Calf	27	<LOQ	15	4100	210	<LOQ
05-09/03	Calf	43	<LOQ	89	12000	310	14
06-08/01	Juvenile	54	<LOQ	100	12000	660	44
06-13/01	Calf	47	<LOQ	49	6800	400	26
06-13/02B	Calf	24	<LOQ	29	4100	230	21

POP concentrations are reported to two significant figures.

<LOQ- None of the compounds included in the sum were detected above the lowest level of quantitation. HCB (Hexachlorobenzene), HCH (Hexachlorocyclohexane), CHLD (Chlordane compounds), PBDE (Polybrominated diphenylethers).

Only a single report for POP comparison with Irrawaddy dolphins exists in the literature (Kannan et al., 2005), comprising five stranded Irrawaddy dolphins in Chilika Lake, near Orissa, India, in 2000 and 2001. However of these animals only one juvenile was from a

similar age class. On a lipid weight basis, with the sole exception of HCHs, average levels of POPs in the Cambodian animals were several times higher than those reported for the Chilika Lake Irrawaddy dolphins (Table 10) with the Chilika juvenile having 1,100ng/g DDT compared to the Cambodian juvenile having 12,000ng/g DDT, 10 times the levels of the Chilika dolphin. In addition two Cambodian juveniles had DDT levels in excess of that found in the Chilika lake adult dolphin.

**Table 10: Mean concentrations (ng/g, Lipid) of Organochlorine contaminants measured in blubber of Irrawaddy dolphins collected in Chilika Lake, India, 2000-2001**

Class	HCB	$\Sigma$ HCHs	$\Sigma$ CHLDs	$\Sigma$ DDTs	$\Sigma$ PCBs	$\Sigma$ PBDEs
Juveniles (n=1)	3.6	180	1.7	1100	28	1.2
Adults (n=4)	10.6	587.5	21.7	5052	214	8.12

Data from Kannan et al., (2005)/ *Arch Environ Contam Toxicol* 49:415-420

The juvenile from Cambodia had blubber levels of  $\Sigma$ PCBs and  $\Sigma$ DDTs of 660 and 12,000 ng/g lipid, respectively, compared to 28 and 1100 ng/g lipid in the Chilika Lake juvenile. Average levels of contaminants for all of the Cambodian animals (n=5, calves + juvenile) were higher than the average levels in Chilika Lake adult dolphins (n=4) (e.g.,  $\Sigma$ DDTs at 7800 vs. 5052 ng/g lipid;  $\Sigma$ PCBs at 360 vs. 214 ng/g lipid;  $\Sigma$ CHLDs at 56 vs. 21.7 ng/g lipid;  $\Sigma$ PBDEs at 21 vs. 8.1 ng/g lipid; HCB at 39 vs. 10.6 ng/g lipid). The only exception to this trend is for  $\Sigma$ HCHs, with no HCHs being present above their lower quantitation limits in the Cambodian samples, whereas levels in Chilika Lake adults averaged 587.5 ng/g lipid.

#### 4.7. Genetic analysis

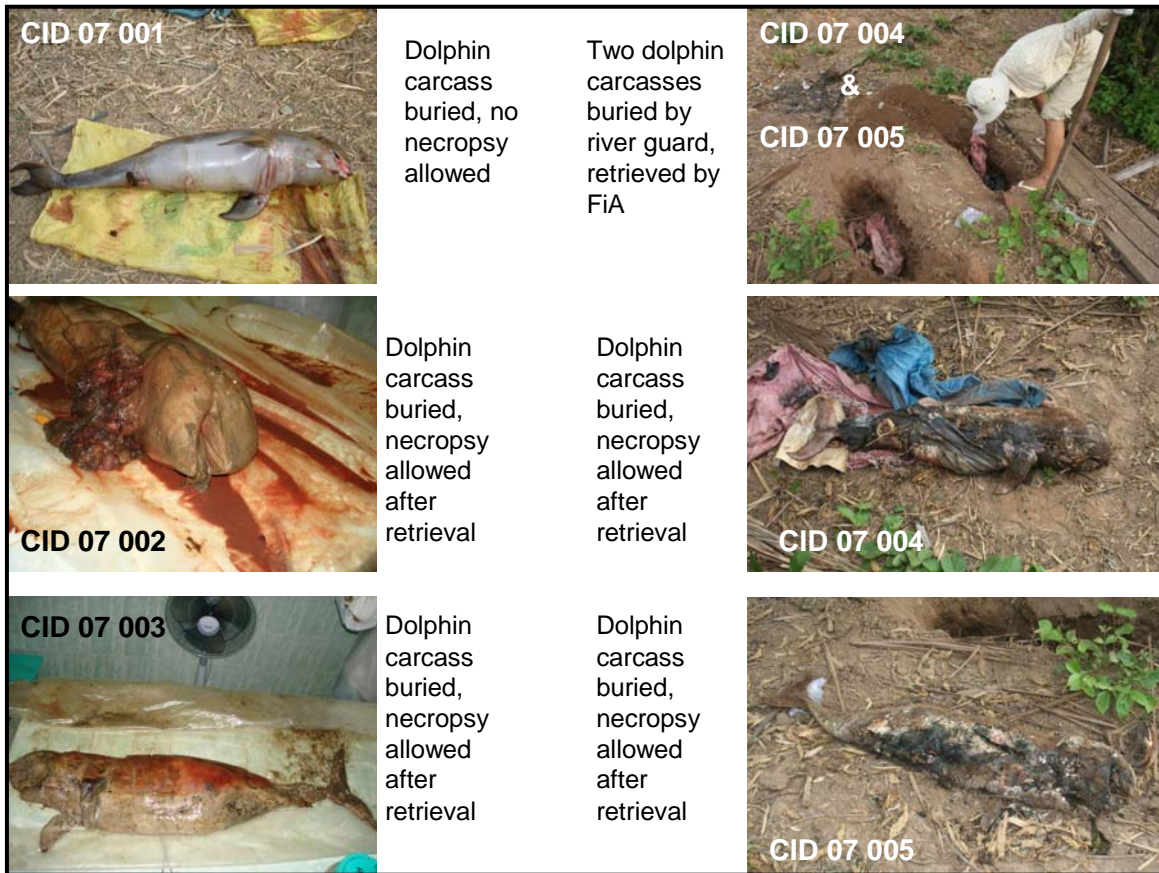
The results of sequencing the mitochondrial control region from 9 Mekong Irrawaddy dolphin samples found that all nine sequences were the same, in that they all have a single haplotype. It was also found that these nine sequences were different in 5 positions out of approximately 800 from an Irrawaddy dolphin sample from Thailand, held at the SWFSC (gene bank) in San Diego, USA.

## **DISCUSSION**

Since mortality studies commenced in 2003, 88 dolphins have died. When evaluated by the number of deaths per year, death counts were the highest in 2006 (n=18) and slightly lower in 2007 (n=14). Of the 88 deaths 65% (n=57) were neonates, 5 % (n=4) were juveniles and 31% (n=27) were adults. When age class was separated out into age in years at death, only animals dying at less than 1 year old were over-represented (n=57). Many wild populations experience highest mortality in the first year age class (Gaydos et al., 2004a, Gaydos et al., 2004b).







From 2003 until 2007, dolphins have died in every month of the year. In 2007- 2009 however there has been a change either in the number of dolphins dying, or the dead dolphins not being reported. In 2007 the mortality rate recorded was 14, lower than previous years. However many dolphin carcasses were not reported to Fisheries or to our project and were buried (See Figure 1), with 8 carcasses being retrieved by the project, so it is plausible that some dolphin carcasses were buried and went unreported, which would explain the discrepancy in the data.

In 2008 the mortality rate was the lowest recorded with only 6 dead carcasses reported. Of these six carcasses permission to carry out necropsy examinations and sample testing was denied in four cases, with the carcass often being withheld from our project for up to one month, before permission was granted for a necropsy examination to be carried out. This has had severe implications with regards to the results obtained. In the cases where the dolphins have been kept on ice or frozen, many bacteria that were present in the dolphins and may have caused the death of the dolphin were killed by this freezing process.



**Figure 1: Some of the 8 dead dolphins that were buried under instruction in 2007**



<p>Carcass condition: when collected</p>	<p>when necropsy permission granted</p>	<p>Carcass was stored on ice and decomposed</p>
 <p>CID 07 013</p>	 <p>CID 07 013</p>	
 <p>CID 07 014</p>	 <p>CID 07 014</p>	<p>Carcass was frozen which limits testing</p>
 <p>CID 08 002</p>	 <p>CID 08 002</p>	<p>Carcass was frozen which limits testing</p>

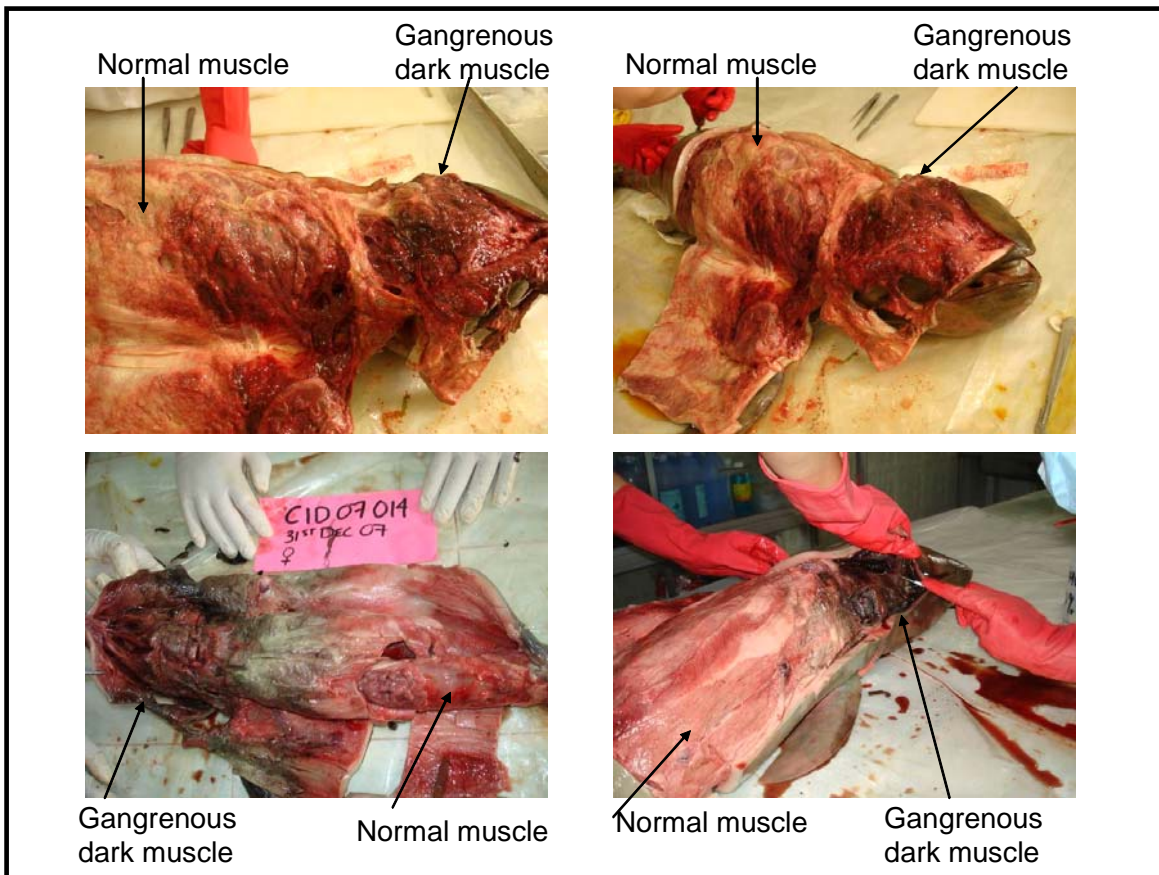
**Figure 2: Some of the dolphins withheld from necropsy examination for up to one month**

#### 4.8. Neck Lesions and Pathogen Screening

During the course of this mortality investigation, the majority of dolphin carcasses presented with a neck lesion that appeared dark purple to blue-black in colouration on macroscopic examination, see Figure 3.



**Figure 3: Neck lesions visible to the naked eye commonly seen in dead dolphins**



**Figure 4: Difference in muscle colour of neck lesion area compared to the rest of the body**

On further examination the underlying soft tissues and muscles were usually wet and swollen, usually gas filled and dark in colouration compared to the muscles of the rest of the body (See Figure 4). To the naked eye the muscles of the neck had a similar appearance to muscles affected by gangrene.

The type of neck lesion seen in the dead dolphins is often referred to as gangrenous necrosis or wet gangrene. This is often a life-threatening condition that occurs when tissue is infected with bacteria, which produce toxins, destroying collagen, which enables the infection to spread rapidly. The infection can spread into the blood stream, to become systemic, this is known as septicaemia. It is unlikely that histopathology alone will reveal the underlying cause of the neck lesions in the dolphins.

As the pathogen screen revealed none of the six pathogens tested for in the dead dolphins, further investigation using an in-country laboratory was deemed appropriate to screen for further microbiological pathogens. Samples from eleven dead dolphins with these characteristic blue/black neck lesions were submitted to Institut Pasteur Du Cambodge, in Phnom Penh for Microbiological testing over the past 2 years. Six of the 11 dolphins had cultures of *Aeromonas hydrophila* either individually or occurring with *Morganella morganii*, or *Pleismonas shingelloides*. Ten dolphins out of the 11 had bacterial infections.

#### ***4.8.1. Aeromonas hydrophila***

A number of dolphin calves and older adults have had cultures with *Aeromonas hydrophila*, which may be a potential cause of the neck lesions and resulting mortality in the Mekong River Irrawaddy dolphin calves. Reports of wounds infected with *Aeromonas hydrophila*, that have fatal outcomes have been increasingly reported in the literature. In addition numerous species are susceptible to the septicaemic effects of this pathogen, with high mortality rates being reported in both immuno-compromised, and immuno-competent hosts. In light of this and the culture results obtained together with the clinical findings in the dead dolphins, it is very likely that *Aeromonas hydrophila* could pose a huge threat to the health of the Mekong Irrawaddy dolphin population.

Motile Aeromonads are among the most abundant bacteria found in freshwater aquatic environments (Camus et al., 1998). Thus river dolphins inhabiting these freshwater environments literally swim in a sea of pathogens (disease causing agents). In particular, bacteria such as *Aeromonas hydrophila* and *Pleismonas shingelloides* thrive in warm freshwater aquatic environments (Tsai et al., 2007). *Aeromonas* are ubiquitous (naturally occurring in the environment) bacteria that are considered primary pathogens that have been implicated in the cause of skin lesions and fatalities of certain freshwater fish species (Angka et al., 1994; Law, 2001) as well as causing gangrene and primary septicaemia in numerous host species (Tsai et al., 2007). Therefore any wound in the normal barrier function of the skin can allow these or other infectious organisms to invade and colonize the skin (Law, 2001).

*A. hydrophila* has been shown to cause a variety of infections and diseases in humans, terrestrial and aquatic mammals, as well as reptiles, birds and fishes. In addition to causing disease in numerous species, several animal species and humans can act as carriers or faecal shedders of *Aeromonas hydrophila*. *Aeromonas* infections are more common in warm water, and infections in fish are most severe in the young fry and fingerlings. Similarly *Aeromonas* causes septicaemia and mortalities in human neonates, infants and children. In addition to affecting the young, *Aeromonas* also causes gangrene and septicaemia in the old, and immuno-compromised individuals. This is the two age classes in the dolphin population that appear to be affected by this bacterium. The bacterium is commonly acquired through an open wound that is exposed to contaminated water. Skin lesions associated with *Aeromonas hydrophila* occur within one day after injury in humans (Tsai et al., 2007), and have a similar appearance to what we are seeing in the dolphin neck lesions. Soft-tissue infections and bacteraemia (bacteria in the blood) with *Aeromonas hydrophila* resemble that caused by clostridia (Borger van der Burg et al., 2006; Furusu et al., 1997) with muscle gangrene and gas production being so severe that mortality rates in humans have been reported as high as 73% (Tsai et al., 2007).

Furusu et al., (1997) proposed two ways to explain how *A.hydrophila* enters the host, which in this study is the dolphin.

1. The bacterium invades through trauma/wound and causes primary infection of soft skin tissue, followed by septicaemia.
2. Septicaemia occurs first induced by *A.hydrophila*, followed by *Aeromonas* seeding infections elsewhere in the body.

It has been reported that some infections caused by *A.hydrophila* in immuno-compromised hosts, have resulted in soft tissue wounds or septicaemia, with no evidence of marked injury or penetrating wound on the body surface, which may have allowed for bacterial invasion (Furusu et al., 1997), and therefore it is possible that both mechanisms could be playing a role in the spread of this disease in these dolphins.

Similar to *Aeromonas hydrophila*, *Pleisiomonas shingelloides* is also a gram-negative facultatively anaerobic rod bacterium, found in the family Vibrionaceae. Similar to

*A. hydrophila*, *Pleisiomonas shingelloides* is also a zoonotic disease, causing bacteraemia, which can result in embolic spread to many organs, in immuno-compromised hosts (Greene, 2006). This water and soil associated organism is found in temperate and tropical freshwater environments, and is prevalent in the intestinal tract of aquatic animals thus infection may be acquired via ingestion of water or fish, shellfish or other aquatic organisms (Greene, 2006).

#### **4.8.1.1. Immunosuppression a potential risk for *Aeromonas* infection**

Stress factors however are thought to be conducive to the rapid proliferation of these bacteria, thereby facilitating the disease, which is thought to have resulted in a mass mortality of many species. In addition stress results in the release of excess cortisol, a hormone that has been implicated in immunosuppression and skin lesions in fish, and may also play a role in the pathogenesis of *Aeromonad* infections in dolphin calves. In addition to stress, a study in which toads were exposed to certain insecticides and then challenged with a sublethal dose of *A. hydrophila*, developed clinical disease, hepatomegaly and died at a higher rate than toads challenged with the same dose of *A. hydrophila*, but not exposed to the insecticide. Thus it may be reasonable to assume that animals exposed to various environmental toxins may develop increased disease susceptibility when challenged with potentially pathogenic bacteria. Decreased nutritional status in individuals may also lead to immunosuppression as well as deficiencies of critical antioxidants which may render the dolphins more susceptible to effects of toxins, pollutants or pathogens within the environment. As numerous reports of *Aeromonas* infections occur in immuno-compromised hosts, all mechanisms for immunosuppression in these dolphins including a lack in genetic diversity, resulting in inbreeding depression, should be considered a potential risk factor for an *Aeromonas hydrophila* outbreak.

#### **4.9. Histopathology Findings**

The histology results rule out trauma such as gill net entanglement or other blunt trauma, as the cause of the neck lesions, as no inflammatory changes were seen histologically.



The histology report confirms the presence of bacteria within the tissue cells, and says that these bacteria are likely to be clostridia that came into the body after death, and produced gas. Microbial culture at Pasteur Institute has been negative for clostridial pathogens in all the dolphin specimens cultured in this study, so this is unlikely to be the bacteria present. However when we cultured for clostridia, Pasteur Institute found *A.hydrophila*, indicating that these may be the intracellular bacteria seen on histology.

In light of the findings of *Aeromonas hydrophila* mentioned previously, the histology report is in agreement with this particular disease agent. *Aeromonas hydrophila* has various toxic properties that can cause severe autolysis (breakdown) of tissue and red blood cells in the host. This bacterium is also able to survive in the host by being able to hide from the hosts' immune system and avoid being detected, which results in no inflammatory changes being produced.

In addition this bacterium produces gangrene tissue which would explain the gas bubble formation histologically and the bruising of the neck visible to the naked eye. All these findings of *Aeromonas hydrophila* are consistent with the histology report.

#### 4.10. Heavy Metal Analysis

**Table 11: Comparison of various mercury levels**

Species	Mercury Concentration (ppm)
	1 ppm = 1 µg/g
Cambodian Fish	0.01-0.96
Mekong Irrawaddy Dolphins	0.03-1.54
Toxic levels: in Humans	0.2
Immuno-toxic levels: in Dolphins	1.0

The mercury levels found in the dolphin samples ranged from 0.03 µg/g –1.54 µg/g, which is higher than the levels found in the fish in Cambodia, and is higher than the minimum level of methylmercury toxicity (200ng/ml or 0.2 ppm in blood) in non-pregnant human adults (Rosa and Lehti, 1996). In a study by Agusa et al., (2005) that examined mercury concentrations in marine and freshwater fish from Cambodia, the fish were found have high concentrations of mercury ranging from <0.01 to 0.96 µg/g wet weight, with some fish specimens being harmful for human consumption. 100 percent of the mercury in fish is in the highly toxic form of methylmercury (MeHg) (Rosa and Lehti, 1996). Since the river dolphins are at the top of the food chain and their diet is almost exclusively fish, they will consequently accumulate high mercury levels in the organic form as this highly toxic MeHg (Bennette et al., 2001). The source of the mercury in the Mekong River dolphins is thought to be from gold mining activities along the Mekong River.

In a study by Pelliso et al., (2008) an *in vitro* approach was developed to test the effects of five heavy metals on the immune response of bottlenose dolphins. Mercury was found to be the most immuno-toxic metal for these dolphins. The majority of the immune system cells were destroyed at 10ppm (10 µg/g) of mercury, however the lowest concentration of mercury that can impair the function of the immune system was only 1 ppm (1 µg/g) of mercury. 1ppm was also found to start killing the white blood cells by a process known as apoptosis (programmed cell death). Their study concluded that at concentrations of mercury from as low as 1ppm, white blood cell function is reduced which can affect the immune status of the dolphin, with higher concentrations resulting in suppressing the dolphins' immune system. Numerous studies have also reported on high mercury concentrations in dolphins that died of infectious disease, compared to dolphins that died in gillnet entanglements or other trauma (Bennet et al., 2001). These findings support the hypothesis that high levels of Mercury are immuno-toxic, making the host more susceptible to infectious disease. Bennette et al., (2001) found that toxic metals cause immunosuppression in cetaceans, and that the Hg: Se molar ratio was higher in porpoises that succumbed to infectious disease. In this study there appears to be sufficient molar excess of selenium in all of the tissues to support normal levels of selenium-



dependent physiology; therefore, toxic effects of mercury-dependent sequestration of tissue selenium appear very unlikely. The mean Se: Hg molar ratio in this study for liver samples was 3.44 and the Hg: Se ratio was 0.33, thus based on these results, excessive mercury exposure would not be expected to be the only cause of the demise of these dolphins. However the reduction of any functional activities of the dolphins' immune system may cause significant weakness capable of altering the dolphins' resistance to disease (Pellisso et al., 2008) as has been suggested in other cetacean species exposed to various pollutants (Bennette et al., 2001).

Mercury is transferred to the foetus whilst the mother is pregnant, with further mercury transfer occurring after birth, via the milk from the mother to the calf. Therefore it can be assumed that calves being nursed from contaminated dolphins are already starting off their lives with high quantities of toxic methylmercury from both lactation and in utero transfer, and will accumulate increasing amounts from consuming mercury contaminated fish as they get older. The Liver mercury concentrations from three out of the four dolphin calves sampled in Table 4 are above 1ppm (1µg/g), which is considered toxic to the immune system, and thus mercury contamination should be considered a potential threat to the Mekong Irrawaddy River Dolphin calves. These levels of mercury contamination are even more significant given that all four dolphins were neonatal calves, and that mercury accumulation is age-dependant, with higher levels accumulating over time due to bioaccumulation through the food chain.

#### **4.11. Dioxin Analysis**

The dioxin and related compounds such as polychlorinated-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar PCBs are of growing concern due to their toxic effect on dolphins (Tanabe, 2002). Coplanar PCBs are largely retained in cetaceans, whereas PCDDs and PCDFs are principally accumulated in terrestrial animals (Tanabe, 2002). This together with the fact that dolphins are able to rapidly metabolize dioxins, may explain why the results for dioxins and furans in the Mekong Irrawaddy dolphin were within a normal considered range, particularly when Tanabe (2002) reported high concentrations of PCDDs and PCDFs in soil dumping sites in Phnom Penh,

Cambodia with a range of 280-1500 and 210-1100 (TEQ/g dry weight) respectively, some of which exceeded the environmental quality standard (1000 TEQ/g dry weight) set forth by the Japanese government. However it is known that cetaceans accumulate dioxin-like compounds with much higher concentrations than humans, and with high levels found in the Cambodian environment, these animals may be at high risk, with a potential for adverse effects on their health (Tanabe, 2002). As dolphins are able to rapidly metabolize these compounds, sampling of live animals may give us a better indication of the toxin levels in these dolphins.

#### **4.12. Persistent Organochlorine Pesticides (POPs) Analysis**

Several species of dolphin have been affected by uncommon diseases and unusual mass mortalities, with man-made contaminants being suspected as the underlying cause due to their potential to disrupt normal endocrine physiology. In addition dolphins that have been affected with disease or succumbed to mass mortality have shown extremely high concentrations of these contaminants (Tanabe, 2002). The contaminants that are very dangerous for dolphins are those that are very toxic, accumulate in the body, and persist in the environment for long periods of time, examples of these are Persistent Organochlorine Pesticides (POPs) for example DDTs, HCHs, CHLs and PCBs (Tanabe, 2002).

The only reference available for comparing contaminant levels in Irrawaddy dolphin samples is by Kannan, et al., (2005), however, since different age classes are not always directly comparable with respect to tissue contaminant levels, the comparison of POPs between the dolphins in this study and those in Chilika Lake in India should be read with caution. In addition the nutritional status and body condition of an animal can affect the measured tissue concentrations of POPs, thus the concentrations in the samples from this study should be compared to other data with some caution (Debier et al., 2006). For example, younger cetaceans have in some cases been shown to carry higher blubber contaminant levels than some adults (Krahn et al., 2007a) as may have been the case with the Chilika adult dolphins' POP levels found to be lower than two of the Cambodian calves and the juvenile, which may result in inaccurate conclusions being drawn. The cause of death between the Chilika lake dolphins, and the Mekong River dolphins appear

to be different, with Kannan, et al., (2005) reporting that most strandings were as a result of small boat propeller strikes, whereas it appears that the Cambodian neonatal calves may have underlying health issues.

In dolphins, blubber is the main repository for POPs, containing up to 95% of the total body burden of DDT and PCBs, due to its high lipid (fat) content (Tanabe et al., 1981).

The lipid profiles in two of the Cambodian dolphin samples indicated that the samples were moderately decomposed. Despite gross differences in body condition and health status between these groups of animals, it appeared that, on average and with the notable exception of HCHs, levels of POPs in the Cambodian dolphins were several times higher than those reported for Irrawaddy dolphins collected in Chilika Lake in 2000–2001. Similar to that found by Kannan et al., (2005), DDT and its metabolites were the predominant contaminants found in the Mekong Irrawaddy dolphins, with the highest concentration in 2 individuals measuring 12,000 ng/g lipid weight in blubber.

It is not surprising that the levels of HCHs in the Cambodian dolphins, were lower than those found in the Chilika lake dolphins, as several studies conducted in Cambodia have shown low levels of HCHs in: human breast milk (Kunisue et al., 2004); fish (Monirith et al., 1999); and mussels (Monirith et al., 2003)], which are all lower than that found in similar samples in India. The range of lipid values reported by Kannan, et al., (2005) in the animals from India were similar to the range for total lipid measured in the Cambodian animals. Although PBDEs were below the lower limit of quantitation in the sample with the lowest lipid content, there was a higher average concentrations of PBDEs in the Cambodian animals than was reported in the Indian animals, and PBDE levels in the Cambodian animals were similar to those recently measured in a fish-eating cetaceans from the North Pacific (Krahn 2007). PBDEs are still widely used, thus environmental PBDE levels in environmental samples have continued to rise over time, however there is insufficient data to indicate whether the differences in PBDEs between the Indian and Cambodian animals are temporal or geographical.

Although limited information exists on contaminant levels in Irrawaddy dolphins, much literature exists on levels in various other cetaceans. There has been much experimental evidence linking POPs such as PCBs to deleterious effects on reproduction, endocrine homeostasis, and immune function (Schwake et al., 2002). Schwacke et al, (2002) describe a risk assessment approach to predict health risks for cetaceans exposed to PCBs. Their risk assessment indicates a high likelihood that reproductive success primarily in primiparous (female having one offspring) females is severely impaired by chronic exposure to PCBs, with reproductive failure being measured in terms of stillbirths or neonatal mortality, in addition they found that females, of higher parity (had many offspring), which have previously off-loaded a majority of their PCB burden, exhibited a much lower risk.

The PCB levels reported in the Cambodian Irrawaddy dolphins however were significantly less than that found in some oceanic dolphins, with stranded Mediterranean Striped dolphins in 1990 having a mean PCB concentration of 778ppm ( $778 \times 10^3 \text{ ng/g}$ ) lipid basis, and healthy free-ranging dolphins having a mean concentration of 282ppm (Garet et al., 2005). However the levels of PCBs in the Cambodian Irrawaddy dolphins were comparable to that found in pre-natal Harbour porpoise (*Phocoena phocoena*) (Gardner et al., 2007), but lower than that found in post-natal *P. phocoena*. This comparison is interesting as Jepson et al., (1999) found that POPs in the Harbour porpoise were associated with infectious disease mortality. The levels of DDT were much higher in the Cambodian Irrawaddy dolphins with a range of 4100-12000 ng/g when compared to that found by Gardner et al., (2007) in *P. phocoena* that had a range of 2100-6600ng/g wet weight. Gardner et al, (2007) also found POP concentrations in foetal samples, implicating gestational transfer from the mother to the calf, as is believed to be true for the Mekong *O. brevirostris*. The study by Lahvis et al., (1995) demonstrates that PCBs and DDT can suppress immune responses, and render cetaceans more susceptible to viral and bacterial infections. Their study showed that as concentrations of either PCBs or DDT increased in peripheral blood, so lymphocyte proliferation reduced according to an inverse correlation. According to their results a significant reduction in lymphocytic proliferation occurred with 2 dolphins that had DDT blood levels of 22.1 and 24.4 ng/g

respectively. Although circulating levels of DDT and PCBs were not measured in this study, it is important to acknowledge that these contaminants have shown a reduced *in vitro* immune response associated with increasing levels of DDT and PCBs in peripheral blood, at levels far lower than those reported in the blubber samples of the Cambodian dolphins.

#### **4.13. Genetic Analysis**

The results of the genetic analysis suggest that there is relatively little genetic diversity in the Mekong River Irrawaddy dolphin population or populations from which they came. However the fairly substantial difference with the Thai sample suggests that there may be a lot more variation between populations, than within this population.

#### **4.14. Contaminants, Disease and Immunosuppression**

Understanding the relationships between environmental contaminants and their potential affects on the dolphins' immune system and therefore the dolphins' susceptibility to disease is a crucial step in this mortality investigation. Various studies have investigated these relationships. For example, correlations have been obtained between concentrations of certain organochlorines such as DDT and PCBs with reduced immune responses as measured by *in vitro* mitogen-induced proliferation responses of lymphocytes cultures from free-ranging dolphins (Lahvis et al., 1995). Similarly Garet et al., (1995) also found that reduced immune response was correlated with increasing whole blood concentrations of several organic contaminants. In addition, the immuno-toxic effect of mercury was demonstrated in a study by Pellisso et al., (2008). Thus understanding the causes of ill health in ecological systems presents a major challenge to scientists due to these complex relationships, but is necessary in order to identify solutions.

Ostfeld et al., (2002) reminds us that as conservation veterinarians we must constantly be aware of the ecological changes that may facilitate rapid disease transmission, when focused on monitoring an epidemic or epizootic within a population. Thus monitoring of what we consider to be healthy ecological systems is crucial for determining the range of variation, resistance and resilience so that stages of ill health can be detected. For example the studies mentioned by Pellisso et al., (2008) and Lahvis et al., (1995) provide

a basis for explaining more complex processes, which affect the health of dolphins subjected to an aquatic environment in which the concentration of environmental contaminants is such, that it can provoke a loss of immunological resistance when faced with potential infectious agents. Based on this understanding, the preliminary findings in this study may suggest that the Mekong population of *O. brevirostris* is vulnerable to heavy environmental contamination; and adverse health risks, from high levels of POPs and mercury, as they are persistent; toxic; and bioaccumulative. If high POP and mercury levels in *O. brevirostris* are capable of reducing host susceptibility to infectious agents, then pathogens that are not documented to cause severe disease in other odontocetes may be more virulent and/or opportunistic in this population of *O. brevirostris* resulting in the high mortality rate seen in the Mekong Irrawaddy neonatal calves.

Jepson et al., (1999) found that female dolphins had had significantly lower levels of POPs than males due to maternal transfer of POPs to offspring, and that chronic PCB exposure predisposes cetaceans to infectious disease mortality. Additional studies by Aguilar and Borrell (1994) and Borrell et al., (1995) have found that the first calf delivered is likely to receive the greatest maternal transfer of POPs. Thus it could be inferred that the high neonatal mortality in the Mekong Irrawaddy dolphins could be due to high levels of immunosuppressive POPs such as DDT and PCBs, as well as high levels of mercury being maternally transferred to neonates during lactation. This level of perinatal immunosuppression could then result in infectious disease mortality, as may be the case in these dolphins with *Aeromonas hydrophila*. In the study by Jepson et al., (1999) they found that the most common causes of infectious disease mortality were not primary pathogenic agents but rather opportunistic pathogens, which most dolphins are exposed to throughout life. They therefore concluded that these diseases are consistent with what might be expected to occur as a result of contaminant-induced immunosuppression.

A study by Carey et al. (1995) suggested that amphibian mortality does not have to be caused by lethal levels of environmental contaminants in the environment. That sub-lethal environmental changes acting singularly or synergistically, could induce stress in

young that enabled their immune systems to be compromised resulting in fatal infections with opportunistic pathogens, as has been shown in a number of studies with *Aeromonas hydrophila*. Carey et al (1995) also provides a review of fatal *Aeromonas hydrophila* infections in larval and young metamorphosed frogs, where adults in the same population survive. Thus a similar mechanism for fatal infections may be occurring with the neonatal Irrawaddy dolphins in the Mekong River. Sub-lethal levels of POPs, together with sub-lethal levels of mercury may be causing immune system compromise, thus facilitating opportunistic pathogens such as *Aeromonas hydrophila* to result in fatal infections. The young neonatal dolphin calves may be even more vulnerable to the effects of *Aeromonas hydrophila* due to an immature immune system confounded with the deleterious effects that contaminants have on immune function.

#### **4.15. Threats Contributing to the Mekong River Dolphin Population Decline**

The Mekong Irrawaddy dolphins are threatened due to gillnet entanglements as well as the following identified factors:

- 1. Disease**
  - a. *Aeromonas hydrophila*
  - b. Other opportunistic bacterial diseases
- 2. Environmental contaminants**
  - a. POPs (DDT & PCBs)
  - b. Mercury
- 3. Inbreeding depression**
  - a. Low genetic diversity

The following section discusses how these various threats all combine together, resulting in dolphin mortality.

##### **4.15.1. Inbreeding and Contaminants Resulting in Immuno-suppression and Disease**

**Inbreeding:** Many species have become isolated in small populations, which render them vulnerable to environmental catastrophe (Packer, 1992). The Irrawaddy dolphins in the Mekong are an example of a small population that are completely isolated from other members of their species and are therefore at risk of extirpation in the immediate future (Smith and Jefferson, 2002). This is because in general small populations have an

increased likelihood of inbreeding and lower reproductive rates, which can lead to low genetic variability, reduced resilience against disease and pollution, reduced population fitness and elevated extinction risk due to catastrophic events (FOC, 2007).

Inbreeding can result in congenital defects both physical and reproductive. This can manifest as abnormal sperm deformities, with increased infertility and decreased birth rate, but even more worrisome is that inbreeding can result in an animal's immune defence system being weakened, rendering them susceptible to disease (Packer, 1992). In a genetics study carried out on inbreeding in lions (Packer, 1992) it was found that there was a significant loss of genetic variability in the lions' immune defence systems. Such a loss of genetic variability could render a population especially susceptible to an epidemic. In addition it was found that genetic inbreeding was closely correlated with a reduction in reproductive rates, adding to the species decline. Thus close inbreeding has shown to cause significant reduction in reproduction and infant survival (Packer, 1992). Thus as Packer (1992) states "even if an endangered species in a bottleneck can withstand whatever human development may be eating away at its' habitat, it still faces the threat of an epidemic that could well be fatal to the entire population".

**Contaminants:** PCB's are recognized as immuno-depressants, and high levels of these together with other pollutants could significantly reduce the resistance of dolphins to disease. The risk of mortality from infectious disease increases with high exposure to PCBs. There is a 2% increase in infectious disease mortality that occurs with every 1mg/kg increase in blubber PCB concentration, and a doubling of risk occurring at approximately 45mg/kg PCB in lipid. In addition to PCBs there is a relationship that exists between dolphin exposure to other Persistent Organochlorine Pesticides (POPs) such as DDT and mortality due to infectious disease. These environmental contaminants or POPs can be passed down from one generation to the next through lactation (milk from the mother to her calf), and they have been implicated in compromised immune functions resulting in high neonatal mortality rates in dolphins from Hong Kong waters (Parsons, 1995). Disease conditions in dolphins that have been implicated as a



consequence of contamination with POPs and heavy metals include: immune dysfunctions, epizootics, liver disease, reproductive and immunological disorders.

These facts give rise to the concern about the long-term contamination and toxic-effects of POPs in dolphins. Also certain heavy metals including mercury are immuno-toxic (toxic to the dolphin's immune system), resulting in immune suppression. The Liver mercury concentrations from three out of the four dolphin calves sampled in this study were above 1ppm (1µg/g), which is considered immuno-toxic, and thus mercury contamination should be considered a potential threat to the Mekong Irrawaddy River Dolphin calves. These levels of mercury contamination are even more significant given that all four dolphins were neonatal calves.

The PCB levels reported in the Cambodian Irrawaddy dolphins were comparable to that found in pre-natal Harbour porpoise (*Phocoena phocoena*) however the levels of DDT were much higher in the Cambodian Irrawaddy dolphins with a range of 4100-12000 ng/g. This comparison is interesting as POPs in the Harbour porpoise were associated with infectious disease mortality. POP concentrations have also been found in foetal samples, implicating gestational transfer from the mother to the calf, as is believed to be true for the Mekong *O. brevirostris*. PCBs and DDT can suppress immune responses, and render cetaceans more susceptible to viral and bacterial infections with DDT blood levels of 22.1- 24.4 ng/g. Although circulating levels of DDT and PCBs were not measured in this study, it is important to acknowledge that these contaminants have shown a reduced *in vitro* immune response associated with increasing levels of DDT and PCBs in peripheral blood, at levels far lower than those reported in the blubber samples of the Cambodian dolphins.

It is possible that the high levels of environmental chemical contaminants such as PCB's, DDTs, and mercury found in these dolphins cause immuno-suppression either singularly or synergistically (see flow chart in Executive Summary), rendering the Irrawaddy dolphins susceptible to fatal opportunistic bacterial diseases such as *Aeromonas hydrophila*. Thus the bio-accumulation of these immunosuppressive POPs and heavy metals may pose a threat to the health and viability of this dolphin population,

particularly as they are already critically endangered. In addition the maternal transfer of POPs to neonates during lactation may represent a greater immuno-toxic threat than exposure acquired as a juvenile or adult rendering the neonates even more susceptible to infectious disease mortality. This would explain the unusually high mortality rates in neonatal calves within the Mekong dolphin population.

Epidemiological investigations that attempt to assess the relation between POP residues levels, and toxic effects are subjected to a large range of confounding factors. Examples of these confounding factors include: other chemicals of known toxicity (DDT), mercury, infectious agents, and other stressors such as habitat alterations caused by human activities. In addition to these factors, other reasons also aid in increasing the difficulty in interpreting the results, namely: the state of the animal at the time of collection, the treatment of samples between collection and analysis, and the reproductive status of the animal.

The photo-identification data has shown that four juveniles have survived in 2007, and three juveniles survived from 2008. This is promising as only two adults died in 2007, and another two adults died in 2008, indicating some hope for the population. Based on the findings in this study it is likely that these offspring were from multi-parity females that may have already previously “dumped” a large proportion of their toxic load of POPs and mercury to previous offspring, making these juveniles less susceptible to infectious disease and thus able to overcome mortality.

**Disease:** The amplified role of disease as a factor limiting species survival can be traced to anthropogenic changes on a global scale, that have direct and indirect influences on the health of wildlife species. These anthropogenic changes that facilitate disease spread or transmission include: climate change, pollution and land degradation and fragmentation, which increases the opportunity for contact and disease transmission among humans, domestic animals and wildlife. Both infectious and non-infectious diseases are being recognised as an increasing challenge to the conservation of wildlife, with many conservation projects failing to meet their objectives as they did not take disease factors into account. Thus although disease has been recognized as a critical issue for many

decades, it is only recently gaining increasing attention from the wider conservation community.

There are three broad processes affected by ongoing global changes, which have profound implications for wildlife health and conservation, namely: alterations in habitat, shifts in wildlife populations, and the resulting changes in disease ecology. For example, indirect threats may make populations under stress more susceptible to disease outbreaks that otherwise would run their course without risking extinction of an entire species, this may be true of the Mekong Irrawaddy dolphin population. In addition certain global anthropogenic (human) changes that have a direct effect on wildlife persistence, allow opportunities for familiar disease to act on wildlife populations in new ways and situations, allowing completely novel diseases to come into play, which can result in a major cause of decline.

## 5. CONCLUSION

In undertaking this study many potential threats have been revealed that pose a real danger to this declining population on the verge of extinction. Modern ecological science has shown us that if the habitat of cetaceans is protected, then the population can show remarkable resilience in the face of external pressures. However protective measures need to be invoked on the precautionary basis that a potential harmful situation for these cetaceans exists, and not wait until harm is identified to take measures.

Certain elementary facts must be accepted if one is to apply conservation and veterinary skills that will benefit an entire wildlife population. Every wildlife population has a natality rate and a mortality rate, and when these two rates are equal then the population is stable. If there are more deaths in a year than births, then there is a decrease in the population size resulting in a declining population. From the results presented here it seems apparent that ‘neonatal mortality’ is responsible for the declining Mekong Irrawaddy dolphin population, with the underlying threats that have been identified in this study being: infectious disease (*Aeromonas hydrophila*), confounded by contaminant loads of POPs and mercury, as well as genetic inbreeding that may all be causing immuno-suppression. These threats may all be additive or synergistic in their complex relationship to each other, making the overall conservation solutions very difficult. Integrating these health issues as one component of conservation, into policy development, will be crucial to the overall success of this project, to reverse the population decline and save the Mekong River dolphin (*Orcaella brevirostris*) from extinction.

The infectious disease that has been identified in this study appears to be significant and correlates well with the gross pathological findings. This disease may have affected this population due to the immuno-suppressive effects of the various environmental contaminants, which individually may have posed no problem, but together the immuno-suppression may be sufficient to render this species susceptible to infectious disease capable of inducing mortality.

## 6. RECOMMENDATIONS

In light of the findings within this report there is an urgent need for:

- On-going long-term abundance, mortality, and pollution monitoring
- Further investigation of the effects of long-term exposure to environmental contaminants on these riverine cetaceans and their ecosystem is warranted as well as developing better techniques for assessing the impacts of the high level of pollutants that facultative freshwater cetaceans are routinely exposed to in many areas
- Further study on the potential additive or synergistic effects of both toxic metals and POPs, on the health status of the Mekong River Irrawaddy dolphins
- Compare the genetic sequences of the Mekong Irrawaddy dolphins with Irrawaddy dolphins from other river systems in order to build a fairly robust population phylogeny as well as exploring nuclear DNA variation and interpopulational differences
- Enhancing the genetic variation within this population, by firstly mixing the genetics from the isolated population at Cheteal with the rest of the population, and secondly by exploring options of using genetic variation from the other riverine populations of Irrawaddy dolphins. The most feasible way of introducing this genetic variation would be through artificial insemination, using semen collected from the various populations, and establishing a modified captive breeding program
- Reducing or eliminate contaminants at their sources, however this may prove difficult
- A preventative health program is required to manage the disease affected dolphins, in order to minimize mortality
- An endangered species recovery plan to enhance the survivability of this population and minimize the risk of extinction

## 7. GLOSSARY

**Anthropogenic:** Relating to human activities

**Antigenicity:** any substance that can stimulate the production of antibodies and combine specifically with them

**Autolysis:** The breakdown of tissues in the body as a result of enzyme degradation, usual process which occurs after death when the body starts decomposing

**Bacteraemia:** Bacteria occurring in the blood stream

**Bacteria:** small micro-organisms, capable of causing disease in their host

**Carcass:** dead body

**Colonize:** to live on or to live in

**Contaminants:** pollutants found in the environment

**Disease:** an illness, or a sickness resulting in debilitation in the host and may also result in death of the host if severe enough

**Epidemiology:** the study of diseases and agents that cause disease, in relation to the environment, and the host

**Extinction:** all members of a species to die out, extirpation is the more correct terminology for extinction of a population of a species

**Fecundity:** the quality of being fecund; capacity, esp. in female animals, of producing young in great numbers.

**Gangrene:** necrosis or death of soft tissue due to obstructed circulation, usually followed by decomposition and putrefaction.

**Genetics:** The science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment.

**Haemorrhage:** to bleed, or to have blood

**Histopathology:** the study of tissue and cells under a microscope

**Host:** a living animal in which a disease organism can live

**Immune System:** a diffuse, complex network of interacting cells, cell products, and cell-forming tissues that protects the body from pathogens and other foreign substances, destroys infected and malignant cells, and removes cellular debris: the

system includes the thymus, spleen, lymph nodes and lymph tissue, stem cells, white blood cells, antibodies, and lymphokines.

**Immuno-Competent:** To have a fully functional immune system

**Immuno-Compromised:** To have an immune system that does not work properly and is weak and susceptible to disease

**Immuno-Suppression:** to suppress or stop the immune system from functioning

**Immuno-Toxic:** Substance that is toxic to the immune system

**Infectious Diseases:** communicable by infection, as from one dolphin to another or from one part of the body to another

**Inflammation:** Pathology. redness, swelling, pain, tenderness, heat, and disturbed function of an area of the body, esp. as a reaction of tissues to injurious agents.

**Lesion:** any localized, abnormal structural change in the body, redness, wound etc

**Microbiological:** the branch of biology dealing with the structure, function, uses, and modes of existence of microscopic organisms.

**Mortality:** the state or condition of being subject to death

**Necropsy:** the examination of a body after death; post-mortem.

**Neonatal:** a newborn calf

**Pathogen:** any disease-producing agent, especially a virus, bacterium, or other microorganism.

**Pathogenesis:** the production and development of disease.

**Post-Mortem:** the examination of a body after death; necropsy

**Recruitment:** the act or process of recruiting calves into the population

**Reproductive Success:** To reproduce successfully

**Sentinel:** an environmental indicator species

**Septicaemia:** the invasion and persistence of pathogenic bacteria in the blood-stream.

**Susceptible:** capable of being affected

**Synergistic:** acting together to produce a combined effect

**Toxin:** any poison produced by an organism, characterized by antigenicity in certain animals and high molecular weight, and including the bacterial toxins

**Toxicology:** study of toxins

**Trauma:** a body wound or shock produced by sudden physical injury, as from violence or accident.

**Vasculitis:** inflammation of a blood vessel

**Vector:** an insect or other organism that transmits a pathogenic fungus, virus, bacterium, etc.

**Virulent:** Bacteriology. causing clinical symptoms.

**Wound:** an injury, usually involving division of tissue or rupture of the integument or mucous membrane, due to external violence or some mechanical agency rather than disease.



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